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EXPERIMENTAL EVIDENCE ON THE PRODUCTION OF THE MUTANT "ARISTAPEDIA" BY A CHANGE OF DEVELOPMENTAL VELOCITIES

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Received September 16, 1939

THE recessive mutant aristapedia (ss^a) of *Drosophila melanogaster* was first described by BALKASHINA (1929). It represents a remarkable case of homoeosis, in which the bristle on the antenna (arista) is transformed into a homologous organ, a tarsus (figure 1a and 1b). BALKASHINA has shown that in aristapedia flies segmentation of the antennal disk starts at two and one-half days of larval age, at the same time that segmentation of the leg disks begins, while in normal flies the segmentation of the antennal disks does not start until four and one-half days of larval age. GOLDSCHMIDT (1938) tried to explain these data in terms of developmental physiological processes. He assumed that the action of the ss^a gene consists of shifting the initiation of an embryological process to a different point in the time of development. He explained that if an evocator which determines leg segmentation is present in the germ at two and one-half days of larval age all disks in the proper stage of development will react to this stimulus by formation of a tarsus. The antennal disk of normal flies is far behind in differentiation at this time and, therefore, will not react to this evocator. According to BALKASHINA the differentiation of the antennal disk of ss^a starts at two and one-half days. This suggests that the differentiation of the ss^a antennal disk is speeded up and that it is so mature at the time when a "leg evocator" is present that it will react simultaneously with the leg disk in starting tarsal segmentation.

In 1937 two sets of experiments were started by the present author in order to bring forward experimental evidence for this view. The first part of these investigations was based on the consideration that if GOLDSCHMIDT'S explanation is correct, a combination of ss^a with different mutants affecting leg structure should exhibit the mutant effect on the antennae of aristapedia as well as on the legs. On the other hand, mutants influencing the arista should show no effect on aristapedia. The second part of the investigations attempted to prove the existence of a difference in developmental velocities by transplanting ss^a disks into normal larvae and normal antennal disks into ss^a larvae.

A short summary of the results obtained was published recently (BRAUN 1939). A more extended discussion will be given here.

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COMBINATIONS OF *ss*^{a} WITH DIFFERENT LEG AND ANTENNAL MUTANTS

For the crosses involving mutants which affect the leg structure the recessive mutations dachs (*d*) and thickoid (*tkd*) were chosen. They are both located in the second chromosome. Dachs reduces the segments of the tarsus and results in shorter legs which are held close to the body. Thickoid exhibits especially short legs. *tkd* and *d* flies were crossed to *ss*^{a} flies, the factor for which is located in the third chromosome. The stock of *ss*^{a} used exhibits a rather constant appearance of the tarsuslike appendage of the antenna (figure 1b).

First *ss*^{a} and *d* were crossed and the F_1 flies inbred. It was possible to clearly distinguish *ss*^{a} *d* flies from the *ss*^{a} flies by the shortened leglike appendage on the antenna, thus showing the leg effect on the tarsuslike part of the antenna (figure 1c and 1d). However, the double recessive hatched about two days later than the normal flies and the number of homozygous *ss*^{a} *d* flies was actually smaller than 1/16 (243 +, 72 *ss*^{a}, 66 *d*, 13 *ss*^{a}). Most F_2 *ss*^{a} flies from these crosses exhibited a strange modification of the tarsuslike part of the antenna. The proximal half was tarsuslike, while the distal half represented an arista (figure 1e).

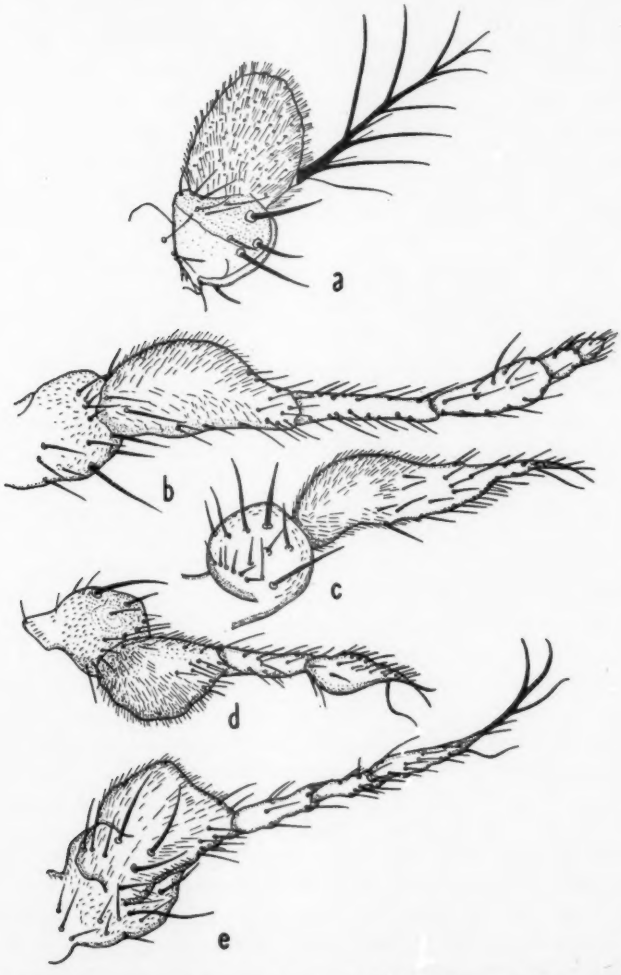
A possible explanation of this strange modification may be suggested. One might assume that a determination stream for aristapedia starts proximally in the antennal anlage of *ss*^{a} and progresses distally in a wavelike fashion. This determination process is interrupted at an early developmental point in these flies due to some as yet undetermined influence of the dachs stock. Therefore, it does not progress completely through the anlage. The distal part of the antennal anlage is thus left unchanged in these particular *ss*^{a} flies and will be determined in subsequent development by the "arista evocator." The result would be as observed, that is, an arista with a proximal leglike base.

This type could be easily selected and was kept as a true-breeding stock for several months before it was accidentally lost. It can be reproduced, however, at any time from a cross of *ss*^{a} and *d* and it deserves some extended and careful investigation.

The homozygous *tkd ss*^{a} flies from the F_2 of a cross between *ss*^{a} and *tkd* also showed a leg effect on the modified tarsuslike arista. These flies hatched only one day later than the normal ones and were more viable than the *ss*^{a} *d* flies of the cross previously described (312 +, 185 *ss*^{a}, 193 *tkd*, 44 *ss*^{a} *tkd*).

These combinations showed convincingly that the modified antenna of *ss*^{a} is affected by the factors which modify leg structure. It now had to be observed whether a factor which influences the normal arista would also change the modified arista of *ss*^{a}. Therefore, in the next set of experiments the mutant aristaless (*al*), which modifies the normal arista, was used. Aristaless is a recessive mutant, located in the second chromosome; it strongly reduces the arista and causes the posterior scutellars to become

erect and divergent. The count of eight bottles of F_2 flies of the cross $ss^a \times al$ gave the following results: 1052 +, 312 ss^a, 264 *al*. Only these three classes were distinguished. However, it was immediately recognized that the excessively large ss^a class probably included the "missing" ss^a *al* flies. This meant that the factor *al* showed no effect on aristapedia flies, at least as regards the altered antennae. In succeeding crosses attention was paid



FIGURES 1a–e.—a, normal antenna; b, antenna of aristapedia; c and d, antennae of "ss^a *d*" flies; e, antenna of ss^a fly from the F_2 of ss^a *d*.

to the posterior scutellars which are modified by the *al* factor. In this way the fourth class "*ss*^{a} *al*" could be distinguished. Four cultures of the F_2 (*ss*^{a} × *al*) gave the following result: 424 normal, 105 aristaless, 115 *ss*^{a}, 50 *ss*^{a} *al*. The "*ss*^{a} *al*" flies showed no effect of *al* on the antennae of aristapedia, but its usual effect on the scutellar bristles was clearly present.

TRANSPLANTATION EXPERIMENTS

Upon the suggestion of DR. R. GOLDSCHMIDT, normal and *ss*^{a} antennal disks of approximately 400 larvae were transplanted into *ss*^{a} and normal larvae respectively. The technique developed by EPHRUSSI and BEADLE (1936) was used. Transplantations were performed between larvae of *ss*^{a} and normal at different stages of larval age from two and one-half days to pupation. The age of the larvae was determined by allowing the mother to lay eggs for 12 hours only. Donor and host were not always of same age. As a preliminary experiment normal antennal disks had been transplanted into normal larvae, in order to check whether the structure of an arista could be observed in a mature transplant. The antennal disks were usually transplanted together with the eye disk to which they are closely attached. This simplified the localization of the transplant in the mature fly. The transplant (when dissected from the adult fly) always presented the appearance of a rather disorderly mass of tissue, bristle, and chitin grown together. In about 30 percent of these control transplants, however, it was possible to locate the arista among the many bristles. The arista of such a transplant was well developed.

Several (nine) transplantations of normal leg disks into normal larvae were performed. No clear segmentation could be observed in the mature transplant. A spiral structure of the width of a tarsus but longer, with bristles on the inside (transplant not everted during development) was the most pronounced structure in the transplant.

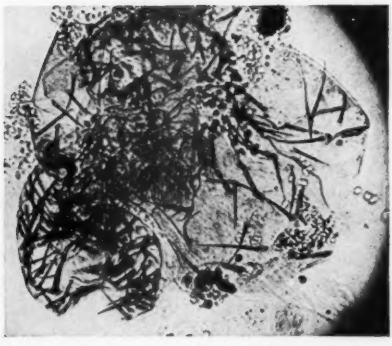
After these preliminary tests were concluded, it was evident that the finding of well-developed aristae in antennal disk transplants and the spiral structure always found in leg transplants would help in analyzing the results of transplantations of disks between *ss*^{a} and normal larvae.

When the antennal disks of normal larvae were transplanted into *ss*^{a} larvae, at stages varying from two and one-half days of larval age to pupation, it was possible to locate a well developed arista in several cases (figure 2a). The spiral structure was never found in these transplants.

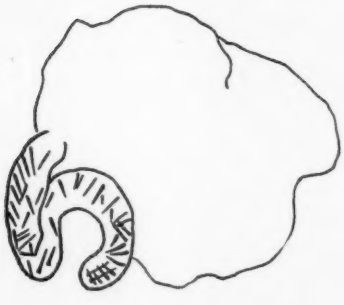
Transplants of *ss*^{a} antennal disks into normal larvae, (again at stages varying from two and one-half days to pupation) always showed the spiral structure, which is assumed to be an undeveloped tarsus (fig. 2b).

Transplantations between larvae younger than two and one-half days

2a



2b



2c

FIGURES 2a, b, c.—a, (above) a mature transplant of normal antennal disk into *ss*[a] larva; b, (center) a mature transplant of *ss*[a] antennal disk into normal larva. Sketch (below) shows location of spiral structure.

were not attempted, since they are extremely difficult on account of the small size of the larvae at this early stage.

DISCUSSION

These results can easily be explained on the basis of GOLDSCHMIDT'S interpretation of the development of aristapedia, and they furnish the first experimental evidence for his views.

It is to be expected that factors influencing the leg structure should also effect the leglike antenna of ss^a if the antennal disk of ss^a starts its differentiation at the same time that the leg disks begin their differentiation. This expectation has been fulfilled by the results of experiments in which the antennal structure of ss^a was observed in combinations of ss^a and different leg mutants.

On the other hand, factors influencing the structure of the arista should not be able to modify the tarsuslike antenna of ss^a. If the antennal disk of ss^a has started its differentiation at the time leg differentiation starts (at two and one-half days of larval age) it will already be far advanced in development at the time when any factors influencing the differentiation of an arista are active in the developing germ (fourth day of larval age). The already differentiated antenna of ss^a, therefore, cannot respond to such factors. This assumption has been proven in the combination of ss^a and a factor influencing the size of the arista; no effect of the aristaless factor could be observed on the antenna in homozygous "ss^a *al*" flies.

What seems at first glance to be a negative result of our transplantation experiments may be additional evidence for accelerated development of the antennal disk in ss^a. The youngest larvae used for our transplantations were somewhat older than two and one-half days, and all ss^a, therefore, had already differentiated antennal disks. The fact that ss^a antennal disks developed into tarsuslike antennae in normal larvae suggests that they may have been differentiated prior to the time of transplantation, namely at two and one-half days of larvae age. The fact that normal antennal disks developed aristae in ss^a larvae does not allow us to draw any conclusions as to the time point of determination for the arista. Determination antedates differentiation and experiments of a different nature have to be carried out in order to find the exact determination point for development of aristae. However, it has to be pointed out that these disks may be autonomous from very early stages and thence transplantation may not test their potencies. The validity of the first interpretation could be checked only if it would be possible to transplant normal antennal disks into a ss^a host prior to two and one-half days of larval age.

From these results we conclude that the ss^a factor acts by shifting the initiation of the differentiation of the antennal disks to an earlier point in

development by speeding up the development of the antennal disks. Thus it happens that the antennal disks of ss^a will be mature enough to respond to a "leg-evocator" by starting tarsus segmentation at a larval age of two and one-half days.

As GOLDSCHMIDT has already pointed out, the same explanation will be valid for other cases of homoeosis, like the mutant proboscipedia (BRIDGES and DOBZHANSKY 1933) and the mutations bithorax and tetraptera (ASTAUROFF 1929). In the latter cases the metathorax exhibits characteristics of the mesothorax; winglike structures are present instead of halteres. In the case of proboscipedia the mouth organs assume the characters of tarsi. The same simple experiments which we used to demonstrate the validity of GOLDSCHMIDT'S explanation for the ss^a case, should also be performed with the mutants just mentioned.

SUMMARY

1. Homozygous combinations of dachs or thickoid with aristapedia showed that the factors influencing leg structure affected the tarsuslike part of the antenna of ss^a flies as well.

2. Homozygous combinations of ss^a with aristaless, a factor influencing the size of the arista and the position of the posterior scutellar bristles showed no effect of aristaless on the tarsuslike part of the antenna of ss^a flies, but its usual effect on the posterior scutellars.

3. Transplantations of ss^a antennal disks into normal larvae at stages from two and one-half days of larval age till pupation resulted in development of structures which were recognized as leglike.

4. Transplantations of normal antennal disks into ss^a larvae at stages from two and one-half days till pupation resulted in development of aristae.

5. These results furnish experimental confirmation of views first expressed by GOLDSCHMIDT concerning the physiology of development of the mutant aristapedia, which acts by shifting the initiation of an embryological process to an earlier point in development.

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INCOMPATIBILITY STUDIES IN COSMOS BIPINNATUS

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Received September 18, 1939

INTRODUCTION

STERILITY phenomena have been studied extensively in many genera—in Antirrhinum by BAUR (1919), FILZER (1926), and SIRKS (1927); in Capsella by SHULL (1929) and by RILEY (1932); in Linaria by CORRENS (1916) and SIRKS (1927); in Nicotiana by EAST (1915a, 1915b, 1918, 1919a, 1919b, 1919c, 1923, 1927), by EAST and PARK (1917, 1918), EAST and MANGELSDORF (1925, 1926) ANDERSON (1924), SIRKS (1927), EAST and YARNELL (1929); in Petunia by TERAO (1923) and by TERAO and U (1929); in Prunus by DORSEY (1919), SUTTON (1918), and CRANE (1925); in Verbascum by SIRKS (1917, 1926, 1927); in Veronica by LEHMAN (1919, 1922) and by FILZER (1926) and others.

But a thorough search of the available literature fails to reveal any reports on sterility in *Cosmos bipinnatus*. In fact, there seems to have been almost no study made on the sterility in any compositae. In the only Cosmos investigations recorded, MIYAKE, IMAI, and TABUCHI (1926, 1927) studied inheritance of flower color, pollen color, and floral characters but made no mention of sterility.

MATERIAL AND METHODS

In the course of breeding new varieties of Cosmos, sterility was first noted when attempts to self F_1 plants repeatedly failed to produce seed.

In 1936 a cross was made between large-flowered pink (from the variety "Sensation") and small-flowered crimson to develop a large-flowered crimson strain. The F_1 was grown in 1937 and consisted entirely of large-flowered crimson-flowered plants, indicating that crimson was dominant over pink, as had been previously recorded by MIYAKE, IMAI, and TABUCHI, and also indicated that large-flowered was dominant over small-flowered. Attempts to self individual plants in the F_1 population showed that they were all self-sterile. Therefore, four plants (well isolated from other Cosmos) were allowed to intercross at random. This produced a normal set of seed.

In 1938 a large F_2 population was grown and this population segregated for flower color and flower size. Crimson, pink, and white-flowered plants were noted, indicating that the original pink-flowered parent was heterozygous for flower color, and suggesting the existence of two factors for

color, one a basic color factor and the other a diluting factor. The segregation in flower size was approximately three large-flowered to one small-flowered. (Of 108 plants, 74 were Crimson, 17 Pink, and 17 White.)

Twenty-five large-flowered crimson plants were selected and numbered and each was individually enclosed in a cheesecloth "cage" to prevent insect pollination (all open flower-heads and seed pods were removed prior to caging). On each plant several heads were left unpollinated as checks, and it was found that none of these unpollinated flower heads produced any seed. This indicated both that all plants were self-sterile and that the cages were effective in preventing any uncontrolled pollination.

After the sterility of all the plants was established, crosses were made between all the 300 possible combinations of the 25 plants.

In Cosmos the ray florets are neutral and all of the disc florets are hermaphroditic. Anthesis of the disc florets occurs centripetally and requires about a week for completion. Emasculation was unnecessary because of their established self-sterility. Pollen was shaken from each of the male parents into an individual sterile glassine bag and in each case it was applied by camel's-hair brush to caged flower heads in which the center disc florets had just opened. At this stage, practically all of the pistils in the disc were receptive.

Within a week or ten days following pollination, its effectiveness could be determined. It was ascertained at once that some combinations were completely fertile while others were more or less completely sterile. The sterile combinations were repeated in order to ascertain whether failure to set seed was the result of inter-sterility or faulty technique in crossing. It is significant that in all cases repetition gave approximately the same negative results.

When the seed ripened, each cross was picked separately and the number of seeds recorded.

(Inasmuch as the work was done primarily to develop a large crimson strain, the 25 selections were backcrossed with a multiple recessive (small-flowered white) to determine the genetic constitution of each. No detailed sterility studies were made in this connection, however.)

RESULTS

As noted above, all 25 selections made in the F_2, as well as the F_1 population, showed complete self-incompatibility.

It was further noted that some combinations were completely cross-fertile, producing nearly a full head of seed, while others were cross-incompatible, producing no seed at all, or, at best, very few seeds.

Moreover, when a plant, A, was incompatible with each of two other plants, B and C, then B and C were also cross-incompatible with each

other. By further studies of the results, it was noted that the 25 selections fell into four intra-incompatible but inter-compatible classes as shown in table 1.

TABLE 1

*Number of seeds set per cross—arranged according to sterility grouping (Failures (under 10) repeated).**

Sel. No.	2	3	6	8	9	10	13	14	18	19	22	1	4	5	7	17	20	21	25	11	15	23	24	12	16
2		1	0	0	0	1	11	0	1	0	1	40	66	80	72	30	73	67	66	67	28	70	78	83	60
3			0	0	1	1	0	0	0	0	3	43	38	62	48	43	41	50	74	43	50	81	76	35	60
6				0	0	0	3	1	2	0	0	78	58	89	64	40	54	72	39	15	59	7	54	54	58
8					0	0	0	0	0	2	0	81	30	64	54	23	32	50	36	61	15	113	40	53	49
9						2	0	8	2	0	3	68	49	67	70	62	51	39	47	61	49	53	55	73	75
10							0	3	2	5	0	21	51	73	70	53	60	39	46	79	38	59	30	52	58
13								1	1	0	0	71	71	52	82	59	75	82	67	88	6	27	00	86	51
14									1	3	0	86	58	37	26	53	101	60	37	23	76	53	63	89	16
18										0	0	30	10	45	30	22	3	5	6	38	56	17	69	58	49
19											0	77	57	50	38	65	64	67	62	69	52	40	40	79	82
22												89	21	70	36	46	27	66	48	89	2	66	47	75	74
1													0	2	5	0	0	1	3	78	36	61	56	45	66
4														0	2	0	9	0	4	60	49	33	55	45	40
5															2	0	0	2	39	106	90	64	70	88	77
7																3	0	5	0	49	49	44	29	39	51
17																	3	0	1	106	7	51	54	81	61
20																		0	1	58	9	67	71	71	42
21																			5	82	62	50	39	72	50
25																				87	50	68	63	65	43
11																					2	4	5	75	81
15																						0	2	90	0
23																							2	92	55
24																								70	66
12																									0
16																									

* In each case where a set of less than 10 seeds was obtained, the cross was repeated. The figures represent combined set in such cases.

Average number of seeds set in compatible crosses— 55.37.

Average number of seeds set in incompatible crosses— 1.79.

Any combination which produced five seeds or less was considered as an incompatible combination. The use of the term "incompatibility" is in no way to be taken to mean the complete failure to set any seed at all, for under certain conditions, such as the end of the flowering period, a few seeds may occasionally be obtained, a manifestation of what EAST (1923) has called "pseudo-fertility." Combinations producing 15 seeds or more were considered as compatible combinations. Those producing 6 to 14 seeds were considered as questionable. On this basis, the results are tabu-

lated as in table 2, incompatibility being represented by a minus sign (−), and compatibility by a plus sign (+), with the questionable combinations symbolized by a question mark (?). According to this classification, the average number of seeds set by the incompatible combinations is 1.79, while the average number set by the compatible combinations is 55.37. This is more significant when it is taken into consideration that many of the incompatible combinations were repeated and the figure 1.79 thus represents, in many cases, more than one pollinated head of some of the combinations.

TABLE 2

Showing inter-compatibility and intra-incompatibility in Cosmos.

Sel. No.	2	3	6	8	9	10	13	14	18	19	22	1	4	5	7	17	20	21	25	11	15	23	24	12	16
2		−	−	−	−	−	?	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3			−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6				−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	?	+	+	+
8					−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9						−	−	?	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10							−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13								−	−	−	−	+	+	+	+	+	+	+	+	+	?	+	−	+	+
14									−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18										−	−	+	+	+	+	+	−	−	?	+	+	+	+	+	+
19											−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
22												+	+	+	+	+	+	+	+	+	−	+	+	+	+
1													−	−	−	−	−	−	−	+	+	+	+	+	+
4														−	−	−	?	−	−	+	+	+	+	+	+
5															−	−	−	−	+	+	+	+	+	+	+
7																−	−	−	−	+	+	+	+	+	+
17																	−	−	−	+	?	+	+	+	+
20																		−	−	+	?	+	+	+	+
21																			−	+	+	+	+	+	+
25																				+	+	+	+	+	+
11																					−	−	−	+	+
15																						−	−	+	−
23																							−	+	+
24																								+	+
12																									−
16																									

(−)=from none to 5 seeds per cross.
(?) =from 6 to 14 seeds per cross.
(+)=from 15 and up.

DISCUSSION AND CONCLUSION

EAST (1927) and EAST and MANGELSDORF (1925) studied the progeny of reciprocal crosses between two self-incompatible plants in Nicotiana (*N. forgetiana*×*N. alata*) and reported three incompatible classes all

inter-compatible but intra-incompatible. The progeny of a cross between members of any two classes consisted of equal proportions of two of the three, the class to which the female parent belongs not being included. Accordingly, when class X was pollinated by class Y or class Z, class X did not appear among the progeny, it consisting only of classes Y and Z in equal numbers. Three alleles for incompatibility, s_1, s_2, s_3, were assumed to explain these results. The genotypes of the three classes were represented as follows:

class X—s_1s_3
class Y—s_1s_2
class Z—s_2s_3

A plant gives stimulus only to pollen carrying genes other than its own. Therefore, pollen carrying the s_2 factor is the only type which will fertilize s_1s_3 plants (class X), and from such a pollination, half of the progenies are s_1s_2 (class Y) and half are s_2s_3 (class Z).

Further studies by EAST and YARNELL (1929) resulted in the isolation of 15 such alleles.

BAUR (1919), FILZER (1926), and SIRKS (1927) studied sterility in *Antirrhinum hispanicum* and found that the mode of inheritance was similar to that of Nicotiana. Other species in which sterility has been studied and found to be inherited in the same manner as Nicotiana are: *Capsella grandiflora* by SHULL (1929); *Linaria vulgaris* by CORRENS (1916) and SIRKS (1927); *Petunia violacea* by TERAO (1923) and TERAO and U (1929); *Verbascum phoeniceum* by SIRKS (1917, 1926, 1927); *Veronica syriaca* by LEHMAN (1919, 1922) and FILZER (1926); and others.

Our data seem to indicate that incompatibility in Cosmos is inherited in a manner similar to that in Nicotiana. Since 23 of 25 plants fell into three groups, we assume three alleles, s_1, s_2, and s_3, to be present in our population, and that the constitution of the three groups were s_1s_2, s_1s_3, and s_2s_3; and that a plant gives stimulus only to pollen carrying genes other than its own, and further that probably a fourth gene, s_4, was present in the remaining two plants, since they were compatible with the other 23 plants but not with each other.

According to the Nicotiana scheme, when the original parents of a controlled cross (not reciprocal) have three alleles, one allele in common, the genotype of the female parent does not recur in the F_1, but if the F_1 plants are allowed to intercross at random, it occurs in half the plants in the F_2, with the other two combinations each making up one-fourth of the population. Consequently, we should expect in the F_2 50 percent of the s_1s_2 (female parent) and 25 percent each of s_1s_3 and s_2s_3 plants.

In our investigation of 23 plants, 11 fell in one group, 8 in another, and

4 in the third group. On the suggested scheme, the expectations would have been 11.5 in the one group and 5.75 in each of the other two.

However, since the F_1 population was small, it is highly possible that instead of two plants each (that is, equal numbers) of s_1s_3 and s_2s_3, there were instead three s_1s_3 plants and one s_2s_3 plant. Such proportions would give in the F_2 11.5 s_1s_2, 8.625 s_2s_3, and 2.875 s_1s_3. This expectancy is more in harmony with the observed data.

The possibilities of the original parents differing by two genes is precluded by the existence of only three certain groups in the F_2. Four sterility factors would give in the F_2 six classes, a condition which did not occur.

The fourth group of two plants was assumed to carry a factor, s_4, since it was compatible with all the plants in the other three groups. The s_4 factor probably was not present in the original parents of the cross. It could easily have been introduced through contamination in the F_1, since the F_1 population was open pollinated.

When the F_2 selections were back-crossed with small-flowered white plants in unrelated lines, plants were found which were compatible with all 25 selections. This indicates the existence of at least a fifth factor for sterility.

Further investigations are being conducted to study the behavior of the various combinations in the third generation.

SUMMARY

1. *Cosmos bipinnatus* is entirely self-sterile.

2. Some plants are inter-fertile while others are inter-sterile.

3. The existence of four allelomorphs for cross-sterility has been established; and the back-cross data indicate the presence of at least an additional fifth factor.

4. Any plant gives stimulus only to pollen carrying genes other than its own, and plants of the same genotype are cross-incompatible.

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A STUDY OF THE EFFECTS OF A RANDOM GROUP OF GENES ON SHAPE OF SPERMATHECA IN DROSOPHILA MELANOGASTER

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WHETHER genes in general affect only one or a few characters of the organism, or whether most genes affect most characters is one of the fundamental problems in physiological genetics.

The consensus in the years immediately following rediscovery of Mendelian heredity was the simple concept of a one-to-one relationship between gene and character. However, instances of dual and even triple effects soon came to light; indeed, MENDEL himself (DOBZHANSKY 1927) pointed out one such effect in the pea. This led to a revision of the general view to include the possibility that a single gene might often affect multiple characters not obviously traceable to a single primary effect.

Later, it was generally recognized among Drosophila workers, that a viability lower than that of the wiid type characterized most laboratory stocks containing mutations. GONZALEZ (1923) supplied quantitative data on this point, validating the general observation. However, these viability differences could not, positively, be ascribed to the single gene which caused the visible effect for which the stock was named, since there was no attempt to make the compared stocks similar for all other loci. Since then TIMOFEEFF-RESSOVSKY (1934 a, b; 1935) has tested the viability reactions of six stocks in which different single mutations were believed to be on a common genetic background. His results are positive. For three reasons, however, these positive results do not provide a basis for assuming the universality of manifold gene effects. In the first place, as HALDANE (1935) has pointed out, the commonality of genetic background is questionable since TIMOFEEFF-RESSOVSKY employed only ten backcrosses between wild type and mutant. In the second place, there is a question whether a sufficient variety of environments was tested and statistically evaluated to eliminate such environmental differences as the cause of the viability differences. Third, and most important, is the strong probability that general viability and fertility effects accompanying a single visible effect might well be the result, not of another gene effect, but of a single, fundamental action basic to both characters.

In 1927, DOBZHANSKY published the first systematic investigation of the problem, employing, not general viability or fertility effects, but a specific, quantitative character. This character was shape of the sper-

matheca in the female *Drosophila melanogaster*. Twelve mutant stocks of *melanogaster* were chosen at random, then backcrossed to wild type a number of times. After backcrossing, the spermathecal index of mutant females was compared with that of their wild-type sisters. Of the 12 stocks, ten showed significant differences in spermathecal index between mutant and wild-type individuals, a difference which DOBZHANSKY felt could be ascribed to the genes whose visible effects marked and named the stocks.

HERSH (1929) made a similar study, using facet number in a Bar-forked stock as his quantitative character. He chose the genes scute, echinus, crossveinless, cut, vermilion and garnet, and studied their effects upon facet number both singly and in combination. Taken singly, scute and echinus were found to have negligible effects; crossveinless and vermilion were strong plus modifiers; garnet and cut were strong minus modifiers. Since the experiments were carried only to the first backcross generation, there is no assurance that the modifying effects upon Bar eye were not due to closely linked genes instead of those causing the marker effects. However, these experiments have given some support to the idea that all genes may affect all characters.

Favoring a negative conclusion is the work of WARREN (1924). Examining egg size in several mutant stocks of *Drosophila melanogaster*, he found this character to be relatively constant for each stock, apparently genetic. However, his investigation indicated that egg size was a function of many genetic factors, none of which could be identified with the genes producing the visible effects which marked and named the stocks.

The work of GREEN (1931), FELDMAN (1935), CASTLE and others (1936 a, b) and of CASTLE (1938) provides interesting examples of somewhat similar studies among mammals. GREEN found, and FELDMAN and CASTLE corroborated, the fact that the gene for brown coat color in mice was accompanied by a consistent increase in body weight and body length as compared with the black mouse. A similar increase (even larger increase in tail length) accompanied the factor "dilution." Although a thoroughgoing attempt to determine whether this increase is due to the brown and dilution factors themselves, or to closely linked genes (GREEN'S interpretation) has not been feasible to date; the evidence so far leans toward the former interpretation. CASTLE (1938) has tested two other genes (albino and non-agouti) to determine whether or not they too might affect the weight and length character. For these two genes, the results were wholly negative.

There is thus a conflict of evidence at the present time. The difficulties involved in reaching definite conclusions seem to be the following: (1) the difficulty of producing two stocks isogenic except for one locus; (2) the difficulty of identifying any gene effects other than simple, additive ones,

by ordinary statistical treatment of differences; (3) the possibility of unidentified significant changes wrought by obscure environmental differences.

Because the question of the universality of manifold gene effects is both fundamental to physiological genetics and important to the genetical approach to problems of evolution, it was felt that the situation justified a reinvestigation of the problem. Shape of spermatheca in Drosophila was chosen as the character to be studied for quantitative effects as an exceptionally favorable character for this purpose and for the sake of comparison with DOBZHANSKY's previous work. The methods used are by no means original, but in a few places, especially in the statistical analysis of the data, an attempt has been made to profit by the work of others to the extent of introducing techniques designed to overcome (in part) the difficulties named in the preceding paragraph, especially the second and third.

METHODS

Metric. The method used for measurement of the spermathecae is substantially that of DOBZHANSKY. The flies chosen for measurement were removed from the bottles within four days after first emergence of adults, brought to boiling in 20 percent KOH and held there for four minutes. They were then carefully run down through 15 percent, 10 percent, and 5 percent KOH (15 minutes minimum in each) to distilled water.

The spermatheca is a dense, chitinous structure whose shape is roughly that of a derby hat without a brim. The mathematical expression of its shape is derived from two measurements, a long measurement like the diameter of a hat from front to rear, and a short measurement like the depth of crown of a hat. The ratio of these measurements provides an index of the shape of the structure. The greatest difficulty encountered is that of so adjusting the spermatheca to the axis of the microscope that the image seen is an optical section at *right angles* to the line of vision and at the plane of maximum diameter of the spermatheca (that is, the center).

For the actual measurement, not more than three (usually only two) pairs of spermathecae were placed in water on a 15 mm hollow ground slide, covered, placed under the microscope and maneuvered into the proper right angled position. Camera lucida notation was made of the long and short axes of each spermatheca, and these notations later measured with vernier calipers.

Certain precautions must be mentioned. The hollow-ground slide is necessary in order to avoid all chance of depressing the spermathecae and thereby altering the axes. It was found also that the presence of more than three pairs of spermathecae on a slide made it quite possible, by acci-

dent, to measure a member of one pair twice. Further, it was found that very small rotations of the mirror of the camera lucida about its axis, small differences in the placement of the spermathecae on the field of the microscope and small differences in position of the observing eye could make substantial differences in the measurements. For this reason, extra efforts were made for achieving consistency. First, the mirror of the camera lucida was carefully adjusted by means of a stage micrometer until it gave equal projections of a line on the stage micrometer, whether that micrometer were in the plane of tilt of the lucida mirror, or at right angles to it. Second, a pointer was placed in the microscope ocular, and every spermatheca placed with its center at this point for measurement. Third, the relative position of eye and ocular was maintained by means of contact of one part of the author's spectacle frames with a part of the camera lucida. Finally, to avoid any residual systematic error which might result from camera lucida distortion, each spermatheca was measured at whatever position relative to the tilt plane of the mirror chance happened to place it.

The consistency of this method of measurement was then tested. At intervals during routine measurements of experimental flies, a few measurements each day were duplicated on separate sides of a card. It should be noted that these were routine measures, not special ones chosen for testing accuracy. From these paired measures, indices were calculated and compared. The series of 100 differences so derived provide a basis for an estimation of error; the standard deviation of the array of differences may be interpreted as a measure of the consistency of a pair of measures and the $SD/\sqrt{2}$ as a measure of the dependability of one measure; the standard error of the mean of such an array of differences over $\sqrt{2}$ may be interpreted as a measure of the consistency of the mean of an array of indices. The values of these two parameters are:

Consistency of a single index	$.039/\sqrt{2}=.027$
Consistency of a mean of 100 indices	$.004/\sqrt{2}=.003$

Thus, it may be seen that third-place differences of means in an experiment are undependable.

Genetic methods. The method used here is a variation of the technique used by DOBZHANSKY; it consists essentially of the repeated backcrossing of each of eleven one-factor mutant stocks to a standard, wild-type isogenic stock, with measurements of the spermathecal index made before, during, and after extensive backcrossing. The details of the method differ from DOBZHANSKY, first in the use of a prepared, isogenic stock; and second in the scheme of backcrossing used.

The isogenic stock was obtained by an extension of the *ClB* principle

to cover all three major chromosome pairs. A male containing crossover-inhibiting, balanced-lethal factors on chromosomes II and III was first crossed with a *ClB* female. From the offspring, a *single* female was chosen, containing only one wild-type member of each chromosome pair. The other member of each pair carries the lethal-crossover-inhibitor combination, thus:

$$\frac{ClB}{+}\ \frac{+}{+}\ \frac{+}{+} \times \frac{+}{-}\ \frac{Cy}{Pm}\ \frac{C\ Me\ Sb\ C}{H} = \frac{ClB}{+}\ \frac{Cy}{+}\ \frac{C\ Me\ Sb\ C}{+}.$$

This female was then backcrossed to her father, yielding both male and female offspring possessing only one wild-type member of each pair of chromosomes, the other member being, as above, the crossover-inhibitor-lethal chromosome, thus:

$$\frac{ClB}{+}\ \frac{Cy}{+}\ \frac{C\ Me\ Sb\ C}{+} \times \frac{+}{-}\ \frac{Cy}{Pm}\ \frac{C\ Me\ Sb\ C}{H} = \frac{+}{-}\ \frac{Cy}{+}\ \frac{C\ Me\ Sb\ C}{+}$$

and

$$\frac{ClB}{+}\ \frac{Cy}{+}\ \frac{C\ Me\ Sb\ C}{+}.$$

These brothers and sisters were then mated *inter se*, and wild-type flies selected. Thus, a wild-type, isogenic stock is obtained in which all second and third chromosomes derive from a *single* II and *single* III chromosome from the original mother, and the X chromosomes derive from the single X of the original father. Perfect isogeneity is probable, but not certain, for there are two possible slips. In the first place, there may not be total suppression of crossing over. However, the reputation of *Cy* (which bears suppressors for both left and right limbs) is good, and so is that of the chromosome III combination. *ClB*, of course, is the best available balancer for chromosome I. In the second place, no attempt was made to inhibit crossing over in the fourth chromosome, simply because no inversion was available. However, the extreme shortness of chromosome IV itself is a crossing over inhibitor of some value.

The production of such an isogenic stock was surprisingly difficult. In the first attempt, *six* females were taken from the first mating, and each mated for 24 hours with their common father. From these six fecundations, six different brother-sister pairs were obtained and mated. In all six cases, the results were nil: each of the six attempts to produce isogeneity had rendered one or more lethal or semi-lethal genes homozygous. The *ClB* chromosome was then transferred to another stock, and another attempt made. This time, 12 independent attempts were made, each with a different pair of parents, and again, the production of stocks homozygous for

lethal or semi-lethal genes was encountered. However, from the 12, four came through, and from these four the one most fertile and viable stock was chosen.

For the extensive backcrossing of autosomal recessive mutants to this isogenic stock, the following scheme was adopted (the large letters represent wild-type alleles in the isogenic stock):

$$(1)\ a\,a \times A\,A = A\,a$$
$$(2)\ A\,a \times A\,a \quad\ a\,a$$

Thus, a generation which permits insertion of haploid sets of chromosomes from the isogenic stock is followed by a generation in which crossing over between isogenic and mutant-stock chromosomes is permitted. It was planned to follow this scheme for 40 generations, thus providing 20 backcrosses interspersed with 20 crossing over matings. In all cases to follow, the notation indicating number of generations will be the number of actual backcrosses; that is, one-half of the total number of generations.

For sex-linked recessives, a similar pattern was used, thus:

(1) Isogenic female × Mutant male = Heterozygous female

(2) Heterozygous female × Isogenic male = Mutant male

Thus, *each* generation affords an opportunity for insertion of an isogenic half-genome; and each alternate generation gives free play to crossing over. It should be noted that in this case, as well as in the case of autosomal genes, only virgin females obtained by the isolation of single pupae in glass vials were used for mating.

Two new considerations now require attention. They are: (1) to what extent has the isogenic stock been substantially altered by mutations during the 36 months which the experiment has taken? (2) What average length of chromosome will remain attached on either side of the selected mutant after a given number of backcrosses?

To provide an answer to the first question, the isogenic stock, uncontaminated by any crosses, was sampled occasionally during the course of the experiment. The results for the shape index a/b are shown in table 1.

Tree characteristics of these data are relevant. First, there is no evidence of trend in the successive samples. In the light of the values to be shown later for the spermathecal index of mutant stocks, trend is known to be the best indicator of any substantial contamination of the isogenic stock. Second, successive samples show no increase in variability. This too would have resulted from an increased heterozygosity of the isogenic stock. Finally, the variability between the successive samples taken at different times can be compared with the variability between samples mated, bred, and measured at the same time. For such a comparison, a standard deviation of measures taken at different times is required. This has been com-

puted from the first five measures in the preceding table (covering the period from June, 1936, to March, 1937, inclusive), by the following method: (1) the actual variance between the five measures has been calculated by the equation, $S^2 = SV^2 - n\bar{V}^2/N - 1$. (2) Variance of means due to sampling has been calculated from the mean of the squared standard deviations of the five measures. (3) Subtraction of sampling variance from

TABLE 1

Spermatheca shape in successive samples.

DATE	MEAN a/b	S.D.	C	N
6/36	2.015 ± .0174	.1754 ± .0123	8.7	102
7/36	1.886 ± .0123	.1287 ± .0087	6.8	109
9/36	1.905 ± .0180	.1797 ± .0127	9.4	100
12/36	1.962 ± .0180	.1795 ± .0127	9.1	100
3/37	1.859 ± .0105	.1049 ± .0074	5.6	100
7/37*	1.936 ± .0150	.046		10×20
10/37*	1.913 ± .0130	.034		7×20
11/37*	1.936 ± .0160	.040		6×20

* For these three cases, standard deviation as shown is based upon the variance between the means of the several bottles of flies shown under N, rather than upon the variance between flies. That the values obtained by this method are indicative of the same order of variability as in the earlier samples is shown by the following values, calculated by the old method for the July, 1937, sample:

SD = .1237 ± .0062 C = 6.4.

the actual variance gives an estimate of the true variance of the means of samples taken at different times. The values so computed are:

Actual variance	.00394
Sampling variance	−.00024
Estimate of true variance	.00370
Estimate of true SD of means	= .061

Similarly, the actual variance between means of bottles mated at the same time has been estimated from the squared deviations of the last three measures given in the preceding table. This has been corrected by deducting sampling variance as above (but based on 20 flies in each case instead of 100), yielding a corrected SD of means of bottles mated at the same time. These values are:

Actual variance	.00169
Sampling variance	−.00123
Estimate of true variance	.00046
Estimate of true SD	= .021

Application of Fisher's z test to the ratio of the variance between means from samples taken at different times, to that of samples taken at the same time indicates that the difference between these two variances is real (probability is beyond the .01 point). But taking into account the wide range of conditions under which the flies were bred from June, 1936, to March, 1937 (differences in season, climate, temperature, food formula), it is improbable that this difference indicates a substantial contamination of the isogenic stock, especially in the light of the lack of trend, and the relative stability of the variance of individual samples.

Turning now to a consideration of the efficacy of continued backcrossing as a technique for production of a common genetic background in two stocks, HALDANE and BARTLETT (1935) offer solutions to the problem for several different schemes of crossing. For the schemes used in this project, they demonstrate (for both autosomal and sex-linked recessives) that the average length of chromosome remaining on either side of the marker gene is equal to $2/M+1\ (1-.75^{M+1})$ where crossing over does not occur in one sex. The value for this expression has been approximated for certain relevant numbers of backcross generations (M, in the expression above) as shown below:

No. of backcross generations	Average length of chromosome on either side
11	16
14	13
15	13
20	9
24	8

It is obvious from the above that the assumption of one-gene differences, even after twenty backcross generations, is not tenable. Therefore, wherever, in the following pages, conclusions are drawn, such conclusions will refer, not to single genes, but to small segments of a chromosome.

Statistical methods. During the first year of the experiment, the samples chosen for measurement consisted of about 100 flies taken from one or a few bottles. The indices of such a batch were computed, and the mean, standard error and standard deviation of such a batch of 100 calculated. In May, 1937, a thoroughgoing sounding was made, which indicated that the technique was inadequate. The data disclosed the fact that bottle-to-bottle differences due to environment were so frequent that comparisons of mutant flies from one bottle with isogenic flies from another were undependable. It also made clear that the plan of the experiment would have to be modified to make possible the detection of gene actions which involved complex interaction of the genes with the environments present in

different bottles. The use of a single large sample from a single environment permits the detection only of consistent, additive effects.

To provide for these two considerations, all experiments after May, 1937, were made as follows: five or more bottles were bred, each from a mating of a single pair of flies. From the offspring within each bottle, a small, constant number (15–20) of mutant and of type flies were taken.

To question these equal, numerous, small samples statistically, the Student Method of paired comparisons was adopted as a first test. The differences between the means of the mutant and wild-type flies from each bottle were taken as the important values. A mean difference (S[d]/n) and its standard error $\left(SE_d^2 = \frac{Sd^2 - \bar{d}\ S\ d}{n(n-1)}\right)$ (where d is each difference, $\bar{d}$ the mean difference, and n the number of bottles) were computed. The ratio of the mean difference to its standard error is then tested by the usual T test.

By this means, differences of scale due to differences in bottle-environment are eliminated; but still only gene effects which result in a net difference will be detected. Very real genetic effects which involve interaction of the genes in different ways with different bottle environments go undetected. To detect this type of gene effect, and further, to provide as complete a picture as possible, a complete analysis of variance of the data of each final experiment (that is, at the conclusion of the planned number of backcrosses) was made. The methods used for this analysis will be described later, with their results.

RESULTS

The first and most direct attack upon the data consists of a T test, asking, in effect, whether or not, for each experiment, a significant difference between type and mutant flies exists at each stage during repeated backcrossing. However, the application of this test at earlier stages in each experiment is prevented. For in these earlier stages, measurements exist only for mutant flies. In order to supply the deficiency in the data, the best estimate of the mean of the isogenic flies over the whole period of the experiment has been calculated by a weighted average of all measures made. This estimate is $1.927 \pm .027$. The standard error appended here is derived from the true SD of means of samples taken at different times (.061) previously computed under "Genetic Methods." (Computation by the usual formula: $SE^2 = SD^2/N$. N, in this case, is 5.)

Whereas the mean for isogenic flies described above is a grand mean of five means, and therefore has the SE equal to $.061/\sqrt{5}$, the means of mutant flies which are to be compared with this grand mean are means of single samples. Their SE, therefore, is equal to $.061/\sqrt{1}$. From these

two standard errors of means, the standard error of their difference may be computed $SE^2_{diff} = SE_1^2 + SE_2^2$). The value of this standard error of a difference is .067, and has been so entered in table 2 of T tests. Differences carrying this standard error are differences computed from the *estimated* value of the isogenic stock. All other entries in the table are actual data or derivatives of actual data.

Two possible sources of confusion should be noted before examination of this table: (1) Whereas, for the parent, F_2 and later generations, the entry under "MEAN" is the mean index of spermathecae from homozygous mutant flies, for the F_1 generation this entry is for heterozygotes. (2) The mutants ivory and vibrissae derive from a single stock; so do the mutants wavy and cherry; hence, for each of these pairs, the measurements for the parent and first filial generations are identical.

TABLE 2

T test and other data derived from the spermathecal indices at various stages during backcrossing.

EXPERIMENT	MEAN	S.D.	N	MEAN TYPE	MEAN MUTANT	N	MEAN DIFFERENCE	T	P
Spineless									
P Gen.	1.634± .011	.1130	111				.293 ± .067		
F_1 Gen.	1.782± .013	.1358	106				.145 ± .067		
F_2 Gen.	1.793± .014	.1490	108				.134 ± .067		
5th BC*	1.758± .013	.1451	119				.169 ± .067		
10th	1.875± .016	.1277	124				.052 ± .067		
15th				1.8577	1.8976	7×15	.0399± .0337	1.18	.4 −.3
20th				1.9169	1.8791	11×15	.0377± .0202	1.87	.1 −.05
Dumpy									
P Gen.	1.476± .010	.1039	107				.551 ± .067		
F_1 Gen.	1.682± .009	.0944	107				.245 ± .067		
F_2 Gen.	1.588± .012	.1149	100				.339 ± .067		
5th BC*	1.696± .011	.1237	128				.231 ± .067		
10th	1.647± .009	.1045	135				.280 ± .067		
15th				1.8610	1.7715	5×14	.0896± .0173	5.2	.01 −.001
20th				1.9096	1.7540	11×15	.1547± .0162	9.5	.001
Brown									
P Gen.	1.733± .012	.1187	101				.194 ± .067		
F_1 Gen.	1.778± .012	.1145	100				.149 ± .067		
F_2 Gen.	1.668± .010	.0966	100				.259 ± .067		
5th BC*	1.678± .009	.1012	115				.249 ± .067		
10th	1.733± .013	.1286	100				.194 ± .067		
15th				1.8340	1.7748	8×10	.0592± .0132	4.5	.01 −.001
20th				1.9037	1.8987	10×15	.0049± .0234	.12	.9
Ebony									
P Gen.	1.945± .014	.1418	100				−.018 ± .067		
F_1 Gen.	1.849± .015	.1500	100				.078 ± .067		
F_2 Gen.	1.843± .013	.1257	100				.084 ± .067		
5th BC*	1.728± .010	.0974	100				.199 ± .067		
10th				1.9514	1.8539	5×18	.0975± .0134	7.3	.01 −.001
15th				1.9213	1.8373	7×15	.0840± .0136	6.2	.01 −.001
20th				1.9077	1.8743	10×15	.0334± .0136	2.5	.05 −.02
White									
P Gen.	1.706± .010	.0965	100				.221 ± .067		
F_1 Gen.	1.727± .009	.0939	100				.200 ± .067		
F_2 Gen.	1.727± .011	.1068	100				.200 ± .067		
4th BC*	1.738± .014	.1437	100				.189 ± .067		
7th				1.8753	1.8383	5×18	.0365± .0167	2.2	.09
11th				1.9639	1.8916	7×15	.0723± .0243	3.0	.03 −.02
15th				1.9040	1.8580	9×15	.0460± .0174	2.6	.03

TABLE 2—*Continued*

EXPERIMENT	MEAN	S.D.	N	MEAN TYPE	MEAN MUTANT	N	MEAN DIFFERENCE	T	P	
Eosin										
P Gen.	1.544± .008	.0830	100				.383 ± .067			
F_1 Gen.	1.615± .009	.0850	100				.312 ± .067			
F_2 Gen	1.617± .010	.0949	100				.310 ± .067			
4th BC*	1.738± .011	.1129	100				.189 ± .067			
7th				1.8155	1.7223	5×18	.0932± .0110	8.5	.000	
11th				1.8556	1.8253	7×15	.0502± .0159	3.2	.02	
15th				1.8887	1.8781	10×15	.0106± .0450	.2	.9	−.8
Ivory										
P Gen.	1.893± .010	.0979	100				.054 ± .067			
F_1 Gen.	1.929± .010	.1019	100				−.002 ± .067			
F_2 Gen.	1.809± .015	.1463	100				.118 ± .067			
4th BC*				1.9601	1.8266	5×18	.1335± .0309	4.3	.02	−.01
7th				1.8588	1.7629	7×15	.0974± .0254	3.8	.01	
11th				1.8993	1.8505	7×15	.0489± .0242	2.0	.1	
20th				1.8802	1.7989	10×15	.0813± .0138	5.9	.00	
Vibrissae										
P Gen.	1.893± .010	.0979	100				.034 ± .067			
F_1 Gen.	1.929± .010	.1019	100				−.002 ± .067			
F_2 Gen.	1.823± .011	.1109	100				.104 ± .067			
4th BC*				2.0302	1.9328	5×18	.0974± .0308	3.2	.05	−.02
7th				1.8706	1.8102	7×15	.0603± .0225	2.7	.05	−.02
11th				1.7990	1.7579	7×15	.0411± .0112	3.7	.01	
Wavy										
P Gen.	1.478± .010	.1005	100				.449± .067			
F_1 Gen.	1.603± .010	.0970	100				.324 ± .067			
F_2 Gen.				1.7628	1.6970	5×18	.0658± .0435	1.5	.2	
4th BC*				1.8076	1.6930	4×18	.1146± .0232	4.9	.02	−.01
7th				1.9240	1.8226	5×18	.1014± .0239	4.2	.02	−.01
11th				1.8910	1.8407	7×15	.0504± .0130	3.9	.01	
Cherry										
P Gen.	1.478± .010	.1005	100				.449 ± .067			
F_1 Gen.	1.603± .010	.0970	100				.324 ± .067			
F_2 Gen.				1.7292	1.7959	5×18	.0667± .0325	2.1	.1	
4th BC*				1.8132	1.7371	5×18	.0761± .0336	2.3	.1	−.05
7th				1.8636	1.8001	6×15	.0635± .0161	3.9	.02	−.01
11th				1.8869	1.8322	6×15	.0547± .0228	2.4	.05	
20th				1.8786	1.8931	9×15	.0144± .0173	.8	.4	
Cardinal										
P Gen.	1.583± .037	.3680	100				.344 ± .067			
F_1 Gen.	1.719± .041	.4088	100				.208 ± .067			
F_2 Gen.	1.738± .014	.1426	100				.189 ± .067			
5th BC*	1.810± .012	.1207	100				.117 ± .067			
10th				1.9284	1.8909	5×18	−.0460± .0232	2.0	.2	−.1
16th				1.9254	1.8597	6×15	.0658± .0189	3.5	.02	−.01
Total			4261			5504				

* 5th backcross, etc.

An examination of the data in table 2 suggests three categories into which the eleven mutants fall. These are:

1. (Spineless, brown, cardinal, wavy, white, eosin, cherry.) The original parent stocks show a very large original difference in spermathecal indices. As backcrossing progresses, this difference becomes smaller, reaching a point of near insignificance in all but cardinal and wavy. The progressive decrease in the difference suggests that a large number of loci in these

stocks were occupied by genes which altered the spermathecal index, and further, that an effective majority of the modifiers affected the index in one direction only (that is, made it smaller than the index of the wild type) a direction which will be referred to in the future as positive.

2. (Ebony, vibrissae, and ivory.) In these three stocks, the original difference between mutant and wild-type indices is inconsequential. But following continued backcrossing, a significant increment is built up and maintained by ivory, but nearly lost in the other two stocks. The suggestion here is that again many loci modify the spermathecal index, but in such a manner that plus and minus modifiers nearly neutralize each other. During early stages of backcrossing more minus than plus modifiers are sloughed off (more plus than minus modifiers closely linked to ebony and vibrissae?) producing a plus difference in spermathecal index. During further backcrossing, plus modifiers are removed from ebony and vibrissae, bringing the difference between mutant and wild-type indices back to a nearly insignificant value.

3. (Dumpy.) Like category 1, above, the original stocks show a large difference. But unlike the mutants in category 1, a *large* significant part of the original difference remains after extensive backcrossing. The situation here suggests that, although many other loci may have contributed to the original difference between mutant and wildtype, a large part of that difference is due to one or more loci within ± 9.5 crossover units of dumpy, perhaps is due to dumpy itself.

It is also of interest to note the changes which take place in the shape index between the P, F_1 and F_2 generations. The F_1 generation is, of course, not only heterozygous for the marker gene, but also for all the other loci (with respect to the alleles introduced from the isogenic stock). Yet, the F_1 in most cases (and especially in the wavy-cherry stock) displays little tendency to move toward the index of the isogenic stock. The implication is that the shape index is determined by many factors, among which the marker gene is of no greater consequence than those whose presence is not indicated by external effects. This tentative conclusion is supported by the fact that in some of the stocks, F_2 flies, homozygous for the marker gene, do not differ markedly from the F_1 flies which are heterozygous for this gene. This is true of spineless, ebony, white, and eosin.

Confining attention to the final measurement alone, the following summary appears to be justified:

MUTANT STOCK	THE FINAL NET DIFFERENCE IS:
dumpy	significant
wavy	significant
cardinal	significant

MUTANT STOCK	THE FINAL NET DIFFERENCE IS:
vibrissae	significant
ivory	significant
ebony	equivocal
white	equivocal
spineless	equivocal
brown	not significant
eosin	not significant
cherry	not significant

Remembering that the terms, *significant*, *equivocal*, and *not significant* in the above list refer only to net differences due to additive, consistent effects of the genes, we turn now to the statistical method for detection of both simple additive and complex, inconsistent interaction effects.

To describe the methods of computation used in the analysis of variance, we shall take a hypothetical experiment involving ten bottles of flies. From each of the ten bottles, the mean spermathecal index of 15 mutant, and 15 wild-type flies is determined. These data are arranged in a two-row table, allotting a column for each bottle. For each bottle, the mean of the wild-type sample is entered in the top row; the mean of the mutant sample is entered in the bottom row, thus:

ARRANGEMENT OF DATA FOR ANALYSIS OF VARIANCE

t′	t′	t′	t′	t′	t′	t′	t′	t′	t′	T′	$\bar{V}$
t	t	t	t	t	t	t	t	t	t	T	
B	B	B	B	B	B	B	B	B	B		
d	d	d	d	d	d	d	d	d	d		

Each t′ represents the mean of 15 wild-type indices; each t represents the mean of 15 mutant indices from the same bottle. T and T′ represent the means of their respective rows: B represents the mean of its column (bottle mean); and $\bar{V}$ represents the grand mean. Each d represents the difference between type and mutant means for its bottle.

From these data, variance among flies will be estimated in seven ways, whose intercomparisons will provide us with the desired information.

The first of these, to be called the *between type* variance (that is, an estimate of variance among flies, assuming no real difference between types), is computed from T, T′, and $\bar{V}$, by the following equation (N = degrees of freedom, and K = weighting factor):

$$\text{Between type variance} = KS\,(T-\bar{V})^2/N.$$

The values of N and K will be given in the summarizing table. (They differ in the different formulae.)

The second estimate (to be called the *between bottle* variance) is computed from the B's and $\bar{V}$, thus:

$$\text{Between bottle variance} = KS\,(B-\bar{V})^2/N.$$

Description of the third (to be called *interaction* variance) will be deferred momentarily for a description of the fourth (to be called *sub-total* variance). This sub-total variance is computed from t, t′, and $\bar{V}$, thus:

$$\text{Sub-total variance} = KS\,(t-\bar{V})^2/N.$$

The third (interaction) variance, mentioned above, is computed by summing the sums of squares of *between type* and *between bottle* variance, and subtracting this sum from the sum of squares of the sub-total variance; that is:

$$\text{Interaction variance} = \frac{KS(t-\bar{V})^2 - (KS(T-\bar{V})^2 + KS(B-\bar{V})^2)}{N}.$$

The fifth variance (to be known as *individual* variance) is computed not from our tabulated means, but from the values of each index of each individual fly, and the mean (t, or t′) of the group to which it belongs, that is (using i to represent the index of individual flies):

$$\text{Individual variance} = KS(i-t)^2/N.$$

The sixth variance (to be called the *total* variance) is computed from the values of individual flies and the grand mean, thus:

$$\text{Total variance} = KS(i-\bar{V})^2/N.$$

The seventh variance (to be known only as variance 1+3) is computed simply from the sum of the sums of squares of *between type* and *interaction* variance, thus:

$$1+3 \text{ variance} = \frac{KS(T-\bar{V})^2 + \text{interaction sum of squares}}{N}.$$

All of this information, plus the actual values of K and N for each estimate of variance is summarized in table 3.

The meaning of each of these estimates of variance is as follows:

The total sum of squares $S^{2mn}(i-\bar{V})^2$ with 2 mn−1 degrees of freedom can be analyzed into two necessarily independent parts: (No. 5), flies from bottle-type means, $S^nS^{2m}(i-t)^2$, with 2(m−1) degrees of freedom, and (No. 4) bottle-type means from the grand mean, m $S^{2n}(t-\bar{V})^2$, with 2n−1 degrees of freedom. (S^x = summation x times.)

The latter, sum of squares of bottle-type means from the grand mean,

can in turn be analyzed into two necessarily independent parts: (No. 2), bottle means from the grand mean, $2m\ S^{n}(B-\bar{V})^2$ with $n-1$ degrees of freedom, and (No. 7) residual variance, $m\ S^{2n}(t-B)^2$ with n degrees of freedom.

A still further analysis of this residual, No. 7, can be made into two necessarily independent parts. These are No. 1, of type means from the grand mean, $mn\ S^{2}(T-\bar{V})^2$ with one degree of freedom, and No. 3, interaction of type and bottle deviation, $m\ S^{2n}((t-B)-(T-\bar{V}))^2$ with $n-1$ degrees of freedom. (Note that $(T-\bar{V})$ is the mean of $(t-B)$.)

TABLE 3

Summary of analysis of variance.

NO.	NAME	SUM OF SQUARES	VALUE OF K*	DEGREES OF FREEDOM
1	Betw. Type	$KS(T-\bar{V})^2$	$n\times m$	1
2	Between Bottles	$KS(B-\bar{V})^2$	2 m.	9
3	Interaction	#(4) below, minus the sum of Nos. (1) & (2) above.	—	9
4	Sub-total	$KS(t-\bar{V})^2$	m	19
5	Individual	$KSS(i-t)^2$	1	280
6	Total	$KS(i-\bar{V})^2$	1	299
7	1+3	The sum of Nos. (1) and (3) above.	—	10

* n = No. of Bottles. m = No. flies per type-sample (15 in all cases).

The ones of greatest interest for the present problem are Nos. 1, 3, 7, and 5. The latter is merely the individual variance of flies from their bottle-type mean. Some light on the nature of the others is obtained by expressing them in similar terms, namely the difference d between type and mutant means within a bottle, and $\bar{d}$, the mean of such differences for an experiment. The expressions are:

$$\text{(No. 1)} \qquad \text{Between type } mn\,\overset{2}{S}(T-\bar{V})^2 = mn\frac{\bar{d}^2}{2}$$

$$\text{(No. 3)} \quad \text{Interaction } \frac{m\,\overset{2n}{S}((t-B)-(T-\bar{V}))^2}{n-1} = \frac{m\,S(d-\bar{d})^2}{2(n-1)}$$

$$\text{(No. 7)} \qquad \text{Residual } \frac{m\,S(t-B)^2}{n} = \frac{m\,S\,d^2}{2n}.$$

For each final measurement on each mutant used, table 4 summarizes the numerical values of these estimates of the variance.

We may now test these data for whatever significant information they may yield. The principle of the test is as follows: each estimate of variance

(as, for instance, the estimate based upon type means, No. 1) may be considered as a way of determining the variance of flies; if there is no real difference between types, the type means estimate should not differ sigficantly from the estimate based directly upon the individual fly measurements, No. 5. We test the two estimates to determine the probability that the difference from equality may occur by chance. For such a test, Fisher's z (1932) or Snedecor's F (1934) (which is derived from Fisher's z) test may be employed. For convenience, the latter has been chosen.

TABLE 4

Analysis of variance—numerical values.

(Values in parentheses are degrees of freedom.)

MUTANT	ESTIMATE DERIVED FROM			
	BETWEEN TYPES (1)	INTERACTION (3)	WITHIN BOTTLE DIFFS. (7)	INDIVIDUAL DIFFERENCES (5)
spineless	.1191 (1)	.0332 (10)	.0410 (11)	.0137 (308)
dumpy	2.1523 (1)	.0182 (10)	.2122 (11)	.0142 (308)
brown	.0005 (1)	.0361 (9)	.0326 (10)	.0144 (280)
ebony	.0835 (1)	.0139 (9)	.0209 (10)	.0103 (280)
white	.1431 (1)	.0205 (8)	.0341 (9)	.0147 (252)
eosin	.0099 (1)	.0208 (9)	.0197 (10)	.0110 (280)
ivory	.4948 (1)	.0143 (9)	.0624 (10)	.0153 (280)
cherry	.0142 (1)	.0201 (8)	.0195 (9)	.0109 (252)
wavy	.1332 (1)	.0089 (6)	.0266 (7)	.0167 (196)
cardinal	.1948 (1)	.0160 (5)	.0458 (6)	.0174 (168)
vibrissae	.0885 (1)	.0066 (6)	.0183 (7)	.0130 (196)

The test consists simply of determining the following ratio of variances: 1/3, 1/5, 3/5, and 7/5, and finding the probability that, for the appropriate number of degrees of freedom upon which each estimate was based, a random deviation would produce a ratio as great or greater than the one found.

If the variance based upon one degree of freedom (No. 1) is found to deviate from the estimate based upon individual fly measurements to an extent that would occur only once in a hundred trials, we conclude that our original assumption was false; that is, that contrary to our assumption, there *is* a real difference between types. Similarly, we may test estimates (3) and (7) against the estimate from individual differences (No. 5). We may also make a further comparison; of estimate (1) to estimate (3). The meaning of this comparison may be deduced from the values in terms of d of estimate (1) and estimate (3), derived in the preceding paragraphs. Estimate (1) was found to be equal to $\frac{1}{2}$n m $\bar{d}^2$. Estimate (3) was found

to be equal to $\frac{1}{2}m\,S(d-\bar{d})^2/n-1$. The ratio of these two is: $n-1\,\frac{n\bar{d}^2}{S(d-\bar{d})^2}$.

In short, test of estimate (1) against estimate (3) is identical with the Student's T test previously used. This provides an interesting link between the simpler test and the present ones.

The significance or non-significance of each of these four tests (that is, 1/5, 1/3, 3/5, and 7/5) may, to some extent be interpreted separately. If the ratio 1/5 be found to be significantly large, it may reasonably be concluded that one or more bottles of flies have contributed values of the shape index for mutant and wild-type flies of a difference greater than would be expected to occur by chance from the variability of flies within a genetic type within a bottle. Similarly, if the ratio 1/3 be significantly large, a *consistent* difference between types within bottles greater than would be expected by chance from fly variability may be concluded; that is, a regular, additive effect of the genes involved is demonstrated. If the ratio 3/5 be significant, it may be concluded that a real genetic effect involving interaction with bottle environments is present. The ratio, 7/5, is the best single test for the existence of any residual variability within bottles, but does not distinguish between additive and non-additive effects (interaction) of the mutation.

For such individual interpretations of separate ratios, a table (table 5) of the values of the ratios, and the probability of a ratio as large or larger occurring by chance is given. The F column is the actual ratio. Because of the fact that the tables of the distribution of F which are available are limited in their scope, actual values are not given under P. Instead, a plus sign is used to indicate a probability of .01 or smaller; an interrogation mark indicates a probability between .05 and .01; and a zero indicates a probability greater than .05. It should be noted that for ratios less than one, the table of F (table 5) has been entered for the appropriate number of degrees of freedom with the reciprocals of these ratios. None has been found to be improbably smaller than unity.

As has been stated, some information can be derived from inspection separately of the significance of the ratios. However, it is the entire constellation of the significances which, taken as a whole, give the best picture of the kind (if any) of genetic determination of spermatheca shape which is operating in each mutant stock. Because the permutations of four things, where each may have any one of three values (+, ?, or o), is unwieldy, a graphic rather than a tabular representation has been chosen.

The graphic representation in figure 1 has been suggested by PROFESSOR SEWALL WRIGHT. Taking the values of ratio C as abscissa and ratio B as ordinate, the results for any experiment can be shown relative to the

lines which indicate complete randomness (F = 1), and deviations from randomness indicated by lines indicating the values of F when the probabilities are .99, .95, .05, and .01.

Since ratio A can be shown to be equal to ratio B/ratio C, the equiprobable lines for this ratio can be indicated as radiating from the lower left-hand corner. Equiprobable lines for ratio D (which is equivalent to ratio B+(n−1) ratio C/N) can be shown cutting across these from lower right to upper left. Thus, a single point can indicate the significance of the results of an experiment relative to all four ratios.

TABLE 5

F test of estimates of variance.

MUTANT	RATIO A 1/2		RATIO B 1/2		RATIO C 1/2		RATIO D 7/5	
	F	P	F	P	F	P	F	P
Spineless	3.6	o	8.7	+	2.4	+	3.0	+
Dumpy	118.8	+	151.6	+	1.3	o	14.9	+
Brown	.01	o	.03	o	2.5	+	2.3	?
Ebony	6.0	?	8.1	+	1.3	o	2.0	?
White	7.0	?	9.7	+	1.4	o	2.3	?
Eosin	.5	o	.9	o	1.9	o	1.8	o
Ivory	34.6	+	32.4	+	.9	o	4.1	+
Cherry	.7	o	1.3	o	1.8	o	1.8	o
Wavy	15.0	+	8.0	+	.5	o	1.6	o
Cardinal	12.2	?	11.2	+	.9	o	2.6	?
Vibrissae	13.4	+	6.8	+	.5	o	1.4	o

As already noted, the location of this point relative to D gives the best single indication of *any* effect of the mutation, but does not distinguish between additive and non-additive effects. (Indeed, as may be seen from the diagram, high significance in *one* of these may not be revealed in D.) The best indication of an additive effect is given by ratio A (equivalent to Student's T test), while location on the C axis is the best indicator of the presence of interaction. The B scale is principally valuable for confirmation of the conclusion from the A scale in the region where C is improbably small (extreme left).

Two special features of the specific diagram should be noted. First, because of reproduction difficulties it was not found feasible to place the .99 and .95 lines for ratios A and B, since they fall too near the edge of the graph. Second, in a strictly accurate presentation, separate diagrams of this kind for each number of bottles used in an experiment should be utilized. However, the present diagram (laid off for n = 10) would be negligibly different for n = 11 and n = 9. For those experiments containing six

or seven bottles, the results, by chance, have fitted satisfactorily into this diagram. Third, the B value for dumpy is greater than shown.

SUMMARY AND CONCLUSIONS

1. Seven segments of chromosomes show direct, additive effects upon

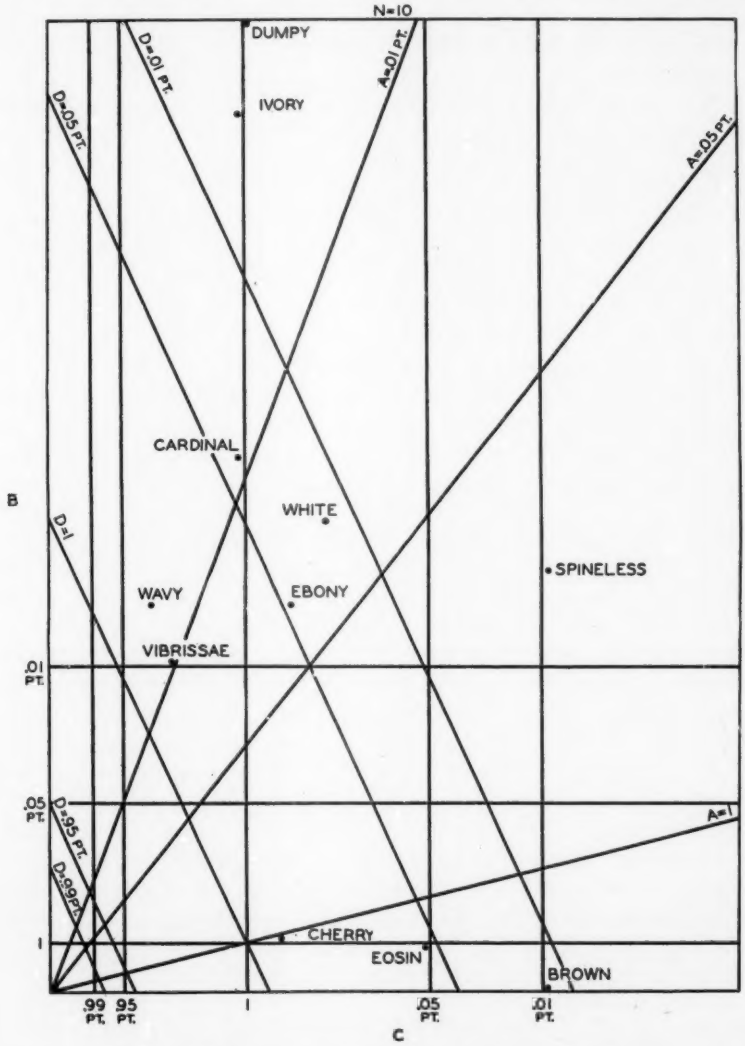

FIGURE 1.—Graphic representation of F tests of estimates of variance.

spermatheca shape. These are: wavy, vibrissae, cardinal, dumpy, white, ivory and ebony. Of these, only dumpy and ivory show a mean difference from the isogenic index of relatively great size. All the others are small.

2. Only one segment of chromosome (brown) shows a complete absence of an additive effect coupled with presence of an interaction effect.

3. One chromosome segment (spineless) shows a strong interaction effect upon spermatheca shape coupled with some evidence of direct, additive effect.

4. Two segments of chromosome (eosin and cherry) show a complete absence of any genetic effect whatsoever.

These results differ in kind from the results achieved by DOBZHANSKY. In twelve stocks tested by him, ten were found to have strong, additive effects. The other two were negative. (These results include, however, several stocks carried through only a few backcross generations.) The results presented here, on the other hand, show only two cases of a *strong* purely additive effect although there are five additional cases of small purely additive effects; and of the remaining four, two show additive effects in stocks also showing interaction effects.

Despite the difference in kind of results, the conclusions based on the data presented here, support, in general, the conclusions of DOBZHANSKY. His paper, written before the appearance of HALDANE'S paper on the value of backcrossing as a producer of isogeneity, assumed apparently that after 20 to 30 generations of backcrossing or inbreeding, residual differences between mutant and wild-type flies could be attributed to the one-gene difference. On that basis, DOBZHANSKY'S tentative conclusion was that there was a high probability that of 12 genes with external, visible effects upon the fly, ten also affected shape of spermatheca. The evidence here presented provides a basis for concluding that, of eleven chromosome segments (containing a gene with external, visible effects) nine are found to contain genes which affect spermatheca shape. Those genes may be one or many; include or exclude the gene with the external, visible effect.

It remains true, nevertheless, that of 11 chromosome segments chosen purely for convenience, nine were found to affect an organ chosen purely for convenience. This is strong evidence for the proposition that many structures are affected by a multiplicity of genes. But for the converse of this proposition, namely that many genes affect a multiplicity of characters, these data offer no evidence. For it cannot be assumed that these ten segments achieve their effect upon spermatheca shape by virtue of the contained gene which affects an external character.

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HANDEDNESS, WITH SPECIAL REFERENCE TO TWINS

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INTRODUCTION

WHILE the occurrence of right-and left-handedness does not appear to conform to any simple Mendelian formula, the familial incidence of left-handedness rather definitely indicates a genetic basis. Children are more likely to be left-handed if one parent is left-handed, than if both are right-handed (CHAMBERLAIN 1928), and in families where *both* parents are left-handed about 50 percent of the children are also left-handed.

Students of twins generally agree (NEWMAN 1928, LAUTERBACH 1925, WILSON and JONES 1934) in finding a higher percentage of left-handedness in both monozygotic and dizygotic twins than in single born populations. This increase is due principally to the frequent occurrence of intra-pair differences in handedness, rather than to any excess of pairs in which both members are left-handed. Both types of twins differ from the single born in that the conditions *in utero* are necessarily changed. But if the position *in utero* is responsible for the differences in the expression of handedness in certain pairs of twins, why does it not affect *all* twin pairs similarly?

NEWMAN (1928, 1937) is of the opinion that in monozygotic twins, embryonic division occurs near or during gastrulation. As the axis of the embryo and bilateral asymmetry are established during gastrulation, he assumes that monozygotic twins which do not separate until after gastrulation will exhibit intra-pair differences in handedness, whereas those separating before gastrulation will be of the same handedness. He also attributes intra-pair variations in other bilateral asymmetrical traits, such as dermatoglyphics, direction of head hair whorl and dentition to the same cause. But if this is the correct explanation, there should be a definite stage in embryonic development for the establishment of each trait showing bilateral asymmetry, beyond which separation of the embryo should result in intra-pair variation, but if separation should occur at some previous time the members of the pair would be alike. Thus we should expect definite combinations of intra-pair trait reversals in monozygotic twins. For example, we might expect to find some twins showing no intra-pair differences in either handedness or direction of hair whorl, some to show reversals in handedness and the same direction of head hair whorl (assuming handedness normally to be established first), some to show differences in both traits, but none to show reversals in hair whorl and the same handedness. Actually, however, we find all possible combinations in re-

spect not only to handedness and hair whorl, but also to any other traits involving bilateral asymmetries. We might logically expect to find *situs inversus viscerum* in one member of monozygotic twin pairs in a high percentage of cases, but actually such cases are extremely rare. Furthermore, Newman's hypothesis completely fails to account for the excess of left-handedness in dizygotic twins.

LAUTERBACH (1925) offers an alternative explanation in which he states "the causes which operate to produce twins also operate to produce left-handed individuals." Considering the quite different modes of origin of the two types of twins, such a hypothesis seems questionable. If true, however, we might expect a higher percentage of left-handers in the non-twin members of the families of twins, than in non-twin families. Such a comparison is included in this paper.

The writer (1938) has observed an apparently high frequency of left-handers among the relatives of twins showing intra-pair variations in handedness, as contrasted with a low frequency of left-handed relatives of twins where both members are right-handed. These observations have suggested a different hypothesis, which could apply to both monozygotic and dizygotic twins. Handedness is assumed to be a quantitative trait, and those individuals who are genotypically intermediate may easily be shifted one way or another in the determination of manual preference. Unusual position *in utero* is one circumstance which may finally condition the handedness of such individuals. The handedness of individuals genotypically strongly right-handed or strongly left-handed, would not be affected by circumstances *in utero*. Thus monozygotic twins showing intra-pair reversals in handedness are assumedly genotypically intermediate in handedness, and the unusual position *in utero* results in one becoming left-handed and the other right-handed. Members of fraternal twin pairs are, of course, of different genotypes, but here, too, the unusual position *in utero* may be sufficient to condition the handedness of genotypically intermediate individuals. A similar explanation for the increase of left-handedness in twins was given by VERSCHUER (1931, 1932).

If the above hypothesis is correct, we should expect to find a higher incidence of left-handedness among the relatives of pairs containing one right-handed and one left-handed member than among the relatives of pairs where both members are right-handed. In this paper we present an analysis of data of our own, as well as that of various other investigators, on the incidence of left-handedness in both twins and the single born.

THE DIAGNOSIS OF HANDEDNESS

There is no general agreement among students of handedness as to just what constitutes right-handedness or left-handedness. The fact that an

association exists between the kicking foot, the dominant eye and the preferred hand has led some investigators (LECHE 1933) to include the kicking foot and the dominant eye among the criteria of handedness. DOWNEY (1927) included both unimanual and bimanual operations in her analyses of handedness. JONES (1918) classified people as to handedness on the basis of arm measurements. NEWMAN (1937) used electrical tapping tests as his criteria for the handedness of twins. Novel tests for handedness, such as blindfolding a person and seeing which way they turn when walking have also been suggested. OJEMANN (1930) in an analysis of artificial tests of handedness such as tapping tests, has shown them to be unreliable. Testimony as to preferred hand, and actual performance appear to be the most reliable tests of handedness. It is hazardous, however, to classify handedness on the performance of only one or two types of tests as has too frequently been done. Writing, for example, is a poor criterion when used alone, in determining the incidence of left-handedness, as so many left-handers have been trained to write with their right hands. When used with other criteria, it is of considerable value, as only rarely do we find right-handers writing with their left hands. Throwing is an excellent criterion for males, but unreliable for females, whereas the hand used for holding a needle is a good criterion for females, and a poor one for males.

In our own classification of handedness, we refer to the hand preferred in the performance of unimanual operations. Our data on an individual's handedness includes his testimony as to whether he considers himself right- or left-handed, or ambidextrous, and the preferred hand for each of the following:[1]

1. Throwing
2. Bowling
3. Marbles
4. Knife
5. Spoon
6. Hammer
7. Saw
8. Sewing
9. Writing
10. Scissors

We have arbitrarily grouped those tested into two classes, right-handers and left-handers. Right-handers include only those who use the right hand for all ten operations, and left-handers those who use the left hand or either hand with equal ease in one or more of the operations.

THE INCIDENCE OF LEFT-HANDEDNESS IN THE GENERAL POPULATION

We recently conducted a survey of the handedness of 687 families. All were families of whom one or more members were students at Ohio State University, taking some course in elementary zoology. Questionnaires

[1] These criteria were suggested by PROF. J. M. RIFE, of Muskingum College.

were prepared asking about the handedness of each member of the family in respect to all items included in our criteria. The students were carefully instructed in the classroom as to how to take the data, and were cautioned not to answer any items concerning which they could not obtain first-hand information. As the questionnaires were given out just before Thanksgiving vacation, the students, with only a few exceptions, had

TABLE 1

The familial occurrence of right- and left-handedness.

TYPE OF CHILDREN	R♂×R♀ (620 MATINGS)	R♂×L♀ (30 MATINGS)	L♂×R♀ (32 MATINGS)	L♂×L♀ (5 MATINGS)
R♂	1084	33	40	2
R♀	758	26	41	3
R♂+♀	1842	59	81	5
L♂	105	6	9	3
L♀	46	10	9	3
L♂+♀	151	16	18	6

opportunities to directly contact the members of their immediate families. Table 1 summarizes the raw data. The excess of males is due to the fact that the great majority of elementary zoology students are males. In a total population of 3542 we find that 263, or 7.45 percent are left-handed. Table 2 shows a close agreement between our findings and those of other investigators.

TABLE 2

Frequencies of left-handedness in the general population.

	NO. OF INDIVIDUALS TESTED	%L
JONES and WILSON	521	6.5
NEALL	800	6.5
QUINAN	1000	7.6
RIFE	3542	7.45

Of the 263 left-handers, 100 are left-handed in all ten operations, and 33 are left-handed in all operations except writing. Twenty-eight are left-handed in only one operation, of whom thirteen are left-handed in throwing, and one is left-handed in writing. No individuals are right-handed only in throwing. Left-handed children occur in families where both parents are right-handed, and five out of a total of eleven children are right-handed in families where both parents are left-handed. These figures indicate the quantitative nature of handedness and clearly show that its inheritance cannot be explained solely on the basis of a single pair of factors.

The fact that left-handed children occur more frequently in families

where one or both parents are left-handed is strikingly shown in the 2×2 table (table 3). Here we find $\chi^2 = 41.7$. As any value of χ^2 beyond 3.841 is considered to be significant, in an analysis involving one degree of freedom, no further comment seems necessary to indicate the tremendous significance of the above differences. It is of interest to remark,

TABLE 3

Two-by-two table showing the incidence of right- and left-handedness among offspring where both parents are right-handed, as contrasted with offspring where one or both parents are left-handed.

	TYPE OF MATING		
	R×R	R×L & L×L	TOTALS
R children	1842	145	1987
L children	151	40	191
Totals	1993	185	2178

$\chi^2 = 41.7$.

however, that our data include a family of five, of which both parents and all three children are left-handed. The children perform all diagnostic operations left-handed, and the parents are left-handed in all except writing. As only about 50 percent of those classed by us as left-handers are as completely left-handed as members of this family, the frequency of the occurrence of such families, if handedness were purely a matter of chance, should be approximately one in a million families of five ($.04^5$).

HANDEDNESS OF TWINS

Numerous investigators have collected data on the incidence of left-handedness in twins. Unfortunately, there has been little uniformity as to criteria used, and some investigators even fail to state any. The writer has obtained data on the handedness of 223 monozygotic and 146 dizygotic pairs of twins. Each pair was tested by the writer and his assistants.

TABLE 4

The frequencies of R-R, R-L and L-L pairs in monozygotic and dizygotic twins.

	MONOZYGOTIC								DIZYGOTIC							
	R-R		R-L		L-L				R-R		R-L		L-L			
	NO.	%	NO.	%	NO.	%	TOTAL	% OF L INDIVIDUALS	NO.	%	NO.	%	NO.	%	TOTAL	% OF L INDIVIDUALS
NEWMAN	30	60	17	34	3	6	50	23	39	78	11	22	0	0	50	11
WILSON & JONES	56	80	13	18.6	1	1.4	70	10.7	97	88.9	24	19.5	2	1.6	123	11.4
RIFE	176	79.5	41	18.3	6	2.2	223	11.35	104	71.3	39	26.7	3	2	146	15.35
Totals	262	76.0	71	20.7	10	3.4	343	14.0	240	75.3	74	23.2	5	1.5	319	13.1

Table 4 is a summary of the data obtained by three American investigators. WILSON and JONES (1934) used throwing and writing as their criteria for handedness. NEWMAN (1937) used tapping tests and testimony as diagnostic criteria. WILSON and JONES' data on monozygotic twins agree closely with our own whereas NEWMAN finds a significantly greater frequency of left-handers in this group. Among dizygotic twins we encountered a higher frequency of left-handedness than did either NEWMAN, or WILSON and JONES. None of the differences in this latter group, however, are statistically significant. All three investigators agree in finding significantly higher percentages of left-handedness in both types of twins than the 7.45 percent obtained in our group of single born individuals.

Table 5 shows the relative frequencies of R-R, R-L and L-L sib pairs in our group of single born individuals, as contrasted with similar groupings for both types of twins. It is apparent that the higher incidence of left-handedness in twins is due principally to the relatively high frequencies of R-L pairs.

TABLE 5

Comparative frequencies of R-R, R-L and L-L pairs in monozygotic twins, dizygotic twins and paired sibs.

	R-R		R-L		L-L	
	No.	%	No.	%	No.	%
Monozygotic	262	76	71	20.7	10	3.4
Dizygotic	240	75.3	74	23.2	5	1.5
Sibs	3067	85.6	475	13.2	41	1.2

THE INCIDENCE OF LEFT-HANDEDNESS IN THE IMMEDIATE FAMILIES OF TWINS

Table 6 summarizes in two-by-two tables our data in regard to the handedness of the immediate relatives of twins. By immediate relatives we refer to parents and sibs. For the identical twins $\chi^2 = 12.8$ and for the fraternal $\chi^2 = 18.1+$. Such values show conclusively that left-handedness occurs more frequently among the immediate relatives of R-L twins, than among the relatives of R-R twins. This is in harmony with what we might expect, if R-L twins are genotypically intermediate in handedness.

Table 7 shows data in two-by-two tables pertaining to the relative frequencies of left-handedness of the single-born in families containing twins, and those containing no twins. Table 7A shows that the incidence of left-handedness is significantly less in the immediate relatives of monozygotic twins, than in non-twin families. This is just the reverse of what we might expect if the same factors are responsible for left-handedness and

twinning, as assumed by LAUTERBACH (1925). We are at a loss, however, to explain why left-handedness should occur with significantly *lower* frequency among the relatives of monozygotic twins. Table 7C shows no significant differences between non-twin families and the families of dizygotic twins in regard to the incidence of left-handedness. Also, when the twins are included, as shown in tables 7B and D, there is no significant

TABLE 6

Two-by-two tables showing the comparative frequencies of left-handed relatives for R-R and R-L twins.

MONOZYGOTIC TWINS

	R-R	R-L	TOTAL
Without L relatives	105	25	130
With L relatives	26	22	48
Total	131	47	178

$\chi^2 = 12.8$

DIZYGOTIC TWINS

	R-R	R-L	TOTAL
Without L relatives	84	12	96
With L relatives	16	15	31
Total	100	27	127

$\chi^2 = 18.1+$

difference in the frequency of left-handedness in non-twin and twin bearing families. These data are apparently not in accord with LAUTERBACH'S explanation of the greater frequency of left-handedness among twins.

SUMMARY

While our criteria for handedness are by no means perfect, and our classification of left-handers is somewhat arbitrary, the data conclusively indicate the following points. Left-handers are more likely to have left-handed children than are right-handers. The inheritance of left-handedness cannot be explained solely on the basis of a single pair of genetic factors. There is considerable evidence that handedness is a graded or quantitative trait. Left-handedness occurs more frequently in both monozygotic and dizygotic twins than in the single born, this being due to the relatively frequent occurrence of pairs of whom one member is right-handed and the other left-handed. In both monozygotic and dizygotic twins, pairs in which one member is left-handed have a significantly higher percentage of left-

handed relatives than do pairs composed only of right-handers. Twin bearing families show no greater incidence of left-handedness than do non-twin bearing families.

The following hypothesis may account for the excess of left-handers among twins. As handedness is a quantitative trait, many individuals are genotypically intermediate, that is, not strongly biased in either direction.

TABLE 7*

Two-by-two tables showing comparative frequencies of left- and right-handedness in twin bearing and non-twin bearing families.

	NON-TWIN FAMILIES	FAMILIES OF IDENTICAL TWINS	TOTALS
Right-handed	3279	978	4257
Left-handed	263	56	319
Totals	**3542** A	**1034**	**4576**

$\chi^2 = 4.9$

	NON-TWIN FAMILIES	FAMILIES OF IDENTICAL TWINS	TOTALS
Right-handed	3279	1128	4407
Left-handed	263	98	361
Totals	**3542** B	**1226**	**4768**

$\chi^2 = .42$

	NON-TWIN FAMILIES	FAMILIES OF FRATERNAL TWINS	TOTALS
Right-handed	3279	509	3788
Left-handed	263	34	297
Totals	**3542** C	**543**	**4085**

$\chi^2 = .90$

	NON-TWIN FAMILIES	FAMILIES OF FRATERNAL TWINS	TOTALS
Right-handed	3279	716	3995
Left-handed	263	63	326
Totals	**3542** D	**779**	**4321**

$\chi^2 = .40$

* In tables A and C, the twins are not included. In B, R-R pairs are included as right-handed individuals and R-L and L-L pairs as left-handed individuals. In D, R-R pairs are included as two right-handed individuals, L-L pairs as two left-handed individuals, and R-L pairs as having one right-hander and one left-hander.

The handedness of such individuals can easily be shifted one way or the other by environmental conditions. In twins, intra-uterine circumstances, such as position and crowding may condition the handedness of such individuals, resulting in one being left-handed and the other right-handed. Individuals genotypically strongly biased for either right- or left-handedness, would not be shifted by environmental circumstances. Thus identical twins genotypically strongly right-handed or left-handed show no intra-pair differences in handedness. Fraternal twins, having different genotypes, may show intra-pair differences in handedness, both on account of

different genotypes and on account of the environmental conditioning of genotypically intermediate individuals. We should expect more left-handedness among the relatives of genotypically intermediate twins than among the relatives of genotypic right-handers. Our data are in harmony with the above conditions.

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CHANCES OF ESTABLISHING THE NON-IDENTITY OF BIOVULAR TWINS, WITH SPECIAL REFERENCE TO INDIVIDUALITY TESTS OF THE BLOOD

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BECAUSE of recent interest in identical and fraternal twins as a means of establishing the hereditary nature of various characters in human beings, there is need for reliable methods of distinguishing the two sorts of twins. In general, if a pair of twins differs in sex or in any other trait, the hereditary nature of which is established, they cannot possibly be monovular. On the other hand, the fact that two twins resemble each other, belong to the same sex and are alike with regard to a large number of hereditary characters, does not absolutely prove them to be monovular, though this would become highly probable.

Aside from sex, the only characters shared by all human beings and transmitted in a simple fashion according to the Mendelian laws are the agglutinogens in the red blood cells. Two of these, designated as A and B, respectively, and discovered by LANDSTEINER in 1900–1901, are transmitted by means of three allelic genes, *A*, *B* and *R* (BERNSTEIN theory) and give rise to four varieties of blood, O, A, B and AB. The existence of two sorts of A agglutinogen, A_1 and A_2, gives rise to subgroups in groups A and AB and increases the number of classes to six. Two additional agglutinogens, M and N, discovered by LANDSTEINER and LEVINE in 1927, are transmitted by a single pair of allelic genes, *M* and *N*, and in combination produce three types of blood, M, N and MN. Since the agglutinogens M and N are independent of A and B, with the aid of the five agglutinogens A_1, A_2, B, M and N, 18 types of human blood can be distinguished. Other agglutinogens have also been described but these are not yet readily available and for a discussion of these, the reader is referred to the review of WIENER (1939).

WIENER (1935) gave the formula for the chance that a pair of biovular twins will differ with respect to the agglutinogens M and N. While the formulas for the other cases were not given, the method of deriving them was indicated. In a recent paper by KOMATU (1939), formulas for the chances of recognizing biovular twins by means of the agglutinogens of human blood are given. All but one of these formulas are incorrect. The correct formula deals with the agglutinogens M and N and agrees with the one previously published by WIENER (1935). It is our purpose to indicate

the errors in the formulas of KOMATU and to give the derivation of the correct formulas.

SINGLE PAIR OF ALLELIC GENES, ONE OF WHICH IS DOMINANT

One of the simplest cases to consider is that of a simple Mendelian dominant, for example, any one of the four agglutinogens A, B, M or N, taken alone.

Let us consider a pair of allelic genes, Q and q, where Q is dominant. These give rise to three genotypes, $Q\,Q$, $Q\,q$, and $q\,q$, the first two corresponding to the type **Q**, and the last one to type **q**. If we take the frequency in the general population of the gene $Q = u$, and the frequency of the gene $q = v$, where $u+v = 1$ or 100 percent, then it is a simple matter to calculate the frequencies of the three genotypes as follows:

$$Q\,Q = u^2$$

$$Q\,q = 2uv$$

$$q\,q = v^2$$

There are six different matings possible and the frequency of each mating in terms of u and v is readily determined by inspection (table 1). In four of the matings all the children belong to a single phenotype, so that in these matings there is no possibility of recognizing fraternal twins. In the mating, $Q\,q \times Q\,q$, $\frac{3}{4}$ of the children belong to type **Q** and $\frac{1}{4}$ to type **q**.

TABLE 1

Chances of recognizing fraternal twins by a character transmitted as a simple Mendelian dominant.

MATING	FREQUENCY OF MATING	FREQUENCY OF CHILDREN OF TYPE		CHANCE OF NON-IDENTITY OF TYPES OF FRATERNAL TWINS
		Q	q	
1) $Q\,Q \times Q\,Q$	u^4	100	0	0
2) $Q\,Q \times Q\,q$	$4u^3v$	100	0	0
3) $Q\,q \times Q\,q$	$4u^2v^2$	75	25	$\frac{3}{4}u^2v^2$
4) $Q\,Q \times q\,q$	$2u^2v^2$	100	0	0
5) $Q\,q \times q\,q$	$4uv^3$	50	50	$2uv^3$
6) $q\,q \times q\,q$	v^4	0	100	0
All Combined	100			$\frac{3}{4}u^2v^2+2uv^3$

The chance that both members of a pair of fraternal twins from such a mating belong to type **Q** is $(\frac{3}{4})^2$ the chance that they both belong to type **q** is $(\frac{1}{4})^2$. Therefore, the chance that two fraternal twins from this mating belong to different types is $1-[(\frac{3}{4})^2+(\frac{1}{4})^2]=\frac{3}{8}$. Since the frequency of the mating is $4u^2v^2$, the contribution of this case to the chance of identifying non-identical twins is $\frac{3}{2}u^2v^2$. Similarly, in the mating $Q\,q \times q\,q$, the chance

that one member of a pair of biovular twins belongs to type **Q** and the other to type **q** is $2uv^3$. Therefore, if P_Q represents the chance, in general, of recognizing fraternal twins with the aid of a simple Mendelian dominant,

Then, $P_Q = \frac{3}{2}u^2v^2 + 2uv^3$

$= \frac{1}{2}uv^2(3u+4v)$.

Since, $u = 1 - v$

$$P_Q = \tfrac{1}{2}uv^2(3+v). \qquad (1)$$

For comparison the formula as reported by KOMATU is given:

$$P_Q = \frac{2uv^2(1+3v)}{(1+v)^2}. \qquad (2)$$

KOMATU does not relate how he arrived at this formula, so that it is not possible to point out the mistake in his derivation. However, as will be shown later, his various formulas, including the one given above, contradict one another, and therefore cannot all be correct.

From equation (1) it is evident that the value of P_Q depends upon the frequencies of the genes (or types). If all the individuals in a population are alike with respect to the trait in question, then either u or $v = 0$, and P_Q has its minimum value, namely, zero. It is of interest to determine the maximum value that P_Q can attain. This is done by setting

$$\frac{dP_Q}{dv} = 0.$$

Since $u = 1 - v$, $P_Q = \frac{1}{2}v^2(3+v)(1-v)$

Therefore, $$\frac{dP_Q}{dv} = \tfrac{1}{2}[6v - 6v^2 - 4v^3] = 0.$$

$2v^2 + 3v - 3 = 0$.

Hence, $v = -\frac{3}{4} + \frac{1}{4}\sqrt{33} = 69.6$ percent.

And $u = 30.4$ percent.

The maximum value of $P_Q = 27.2$ percent.

The distribution of the types for this value of P_Q is as follows:

Q = 51.6 percent and **q** = 48.4 percent.

INDEPENDENT FACTORS

If the chances of recognizing fraternal twins by each of two or more independent hereditary properties is known, then it is a simple matter to calculate the chance of solving this type of problem by the combined use of all the characters. Thus, let $P_1, P_2, P_3 \cdots P_n$ represent the individual chances for n different traits, respectively. The chance that the fraternal

twins resemble each other with regard to every one of these characters is

$$(1-P_1)(1-P_2)(1-P_3)\cdots(1-P_n).$$

Therefore the chance that they differ with regard to at least one character, which is the probability P that we want to derive, is as follows:

$$P = 1-(1-P_1)(1-P_2)(1-P_3)\cdots(1-P_n). \qquad (3)$$

The maximum value of P for n independent Mendelian dominant characteristics is $1-(1-0.272)^n$.

SINGLE PAIR OF ALLELIC GENES, WITHOUT DOMINANCE

As has already been shown, the agglutinogens M and N are transmitted by a pair of alleles, *M* and *N*. Three genotypes are possible, *MM*, *NN* and *MN*, corresponding to types **M**, **N** and **MN**, respectively.

It is a simple matter to derive the value of P_{MN} by using the same method as that given for a simple Mendelian dominant. From table 2, it is evident that

$$P_{MN} = 2m^3n + 2mn^3 + \tfrac{5}{2}m^2n^2. \qquad (4)$$

where m and n represent the frequencies of genes *M* and *N*, respectively. Since $m+n=1$, equation (4) reduces to

TABLE 2

Chances of recognizing fraternal twins by the agglutinogens M and N.

MATING	FREQUENCY OF MATING	FREQUENCY OF CHILDREN OF TYPE			CHANCE OF NON-IDENTITY OF TYPES OF FRATERNAL TWINS
		M	**N**	**MN**	
1) *MM*×*MM*	m^4	100	0	0	0
2) *MM*×*NN*	$2m^2n^2$	0	0	100	0
3) *MM*×*MN*	$4m^3n$	50	0	50	$2m^3n$
4) *NN*×*NN*	n^4	0	100	0	0
5) *NN*×*MN*	$4mn^3$	0	50	50	$2mn^3$
6) *MN*×*MN*	$4m^2n^2$	25	25	50	$\frac{1}{2}m^2n^2$

$$\begin{aligned} P_{MN} &= 2mn(m^2+n^2) + \tfrac{5}{2}m^2n^2 \\ &= 2mn(1-2mn) + \tfrac{5}{2}m^2n^2 \\ &= 2mn - \tfrac{3}{2}m^2n^2 \qquad (5) \\ P_{MN} &= \tfrac{1}{2}mn(4-3mn). \end{aligned}$$

In this case, as in that of a simple Mendelian dominant, the value of P_{MN} becomes zero if the frequency of either gene is zero. Its maximum value is obtained by setting $\frac{dP_{MN}}{dm} = 0$. Since $n = 1-m$,

$$P_{MN} = \tfrac{1}{2}m(1 - m)[4 - 3m(1 - m)]$$

$$\frac{dP_{MN}}{dm} = 2 - 7m + 9m^2 - 6m^3 = 0.$$

Therefore, $(1 - 2m)(2 - 3m + 3m^2) = 0$.

So that $m = \frac{1}{2}$ and $n = \frac{1}{2}$.

Hence P_{MN} has its maximum value when the distribution of the types in the population is **M** = 25 percent, **N** = 25 percent and **MN** = 50 percent, and the maximum value of P_{MN} is $\frac{13}{32}$ or 40.6 percent.

In most white populations thus far examined the frequency (m) of gene *M* is approximately 55 percent; in such populations the value of P_{MN} = 40.3 percent, which is very close to the maximum value.

MULTIPLE ALLELIC GENES

As has been indicated above, the four blood groups furnish an example of heredity by means of multiple allelic genes in man. If p, q and r represent the frequencies of the genes *A*, *B* and *R*, respectively, then, the value of P_{AB} is derived as shown in table 3.

TABLE 3

Chances of recognizing fraternal twins by the four blood groups.

MATING	FREQUENCY OF MATING	FREQUENCY OF CHILDREN OF GROUP				CHANCE OF NON-IDENTITY OF GROUPS OF FRATERNAL TWINS
		O	A	B	AB	
1) *RR*×*RR*	r^4	100	0	0	0	0
2) *RR*×*AA*	$2p^2r^2$	0	100	0	0	0
3) *RR*×*AR*	$4pr^3$	50	50	0	0	$2pr^3$
4) *RR*×*BB*	$2q^2r^2$	0	0	100	0	0
5) *RR*×*BR*	$4qr^3$	50	0	50	0	$2qr^3$
6) *RR*×*AB*	$4pqr^2$	0	50	50	0	$2pqr^2$
7) *AA*×*AA*	p^4	0	100	0	0	0
8) *AA*×*AR*	$4p^3r$	0	100	0	0	0
9) *AA*×*BB*	$2p^2q^2$	0	0	0	100	0
10) *AA*×*BR*	$4p^2qr$	0	50	0	50	$2p^2qr$
11) *AA*×*AB*	$4p^3q$	0	50	0	50	$2p^3q$
12) *AR*×*AR*	$4p^2r^2$	25	75	0	0	$\frac{3}{4}p^2r^2$
13) *AR*×*BB*	$4pq^2r$	0	0	50	50	$2pq^2r$
14) *AR*×*BR*	$8pqr^2$	25	25	25	25	$6pqr^2$
15) *AR*×*AB*	$8p^2qr$	0	50	25	25	$5p^2qr$
16) *BB*×*BB*	q^4	0	0	100	0	0
17) *BB*×*BR*	$4q^3r$	0	0	100	0	0
18) *BB*×*AB*	$4pq^3$	0	0	50	50	$2pq^3$
19) *BR*×*BR*	$4q^2r^2$	25	0	75	0	$\frac{3}{4}q^2r^2$
20) *BR*×*AB*	$8pq^2r$	0	25	50	25	$5pq^2r$
21) *AB*×*AB*	$4p^2q^2$	0	25	25	50	$\frac{1}{2}p^2q^2$

By combining terms, we have

$$P_{AB} = 2pr^3 + 2qr^3 + \tfrac{3}{2}p^2r^2 + \tfrac{3}{2}q^2r^2 + 8pqr^2 + 7p^2qr + 7pq^2r + 2p^3q + 2pq^3 + \tfrac{5}{2}p^2q^2 = r^2(2pr + 2qr + \tfrac{3}{2}p^2 + \tfrac{3}{2}q^2 + 8pq) + pq(7pr + 7qr + 2p^2 + 2q^2 + \tfrac{5}{2}pq). \quad (6)$$

But $p + q + r = 1$.

And $p^2 + q^2 + r^2 + 2pq + 2pr + 2qr = 1$.

Therefore, $P_{AB} = r^2(1+\tfrac{1}{2}p^2 + \tfrac{1}{2}q^2 - r^2 + 6pq) + pq(2 - 2r^2 + 3pr + 3qr - \tfrac{3}{2}pq)$.

$P_{AB} = r^2(1 - r^2) + \tfrac{1}{2}r^2(p^2 + 12pq + q^2) + 2pq(1 - r^2) + \tfrac{1}{2}pq(6pr + 6qr - 3pq)$.

$P_{AB} = r^2(1 - r^2) + \tfrac{1}{2}r^2[(p + q)^2 + 10pq] + 2pq(1 - r^2) + \tfrac{1}{2}pq[6r(p + q + r) - 6r^2 - 3pq]$.

$P_{AB} = r^2(1 - r)(1 + r) + \tfrac{1}{2}r^2(1 - r)^2 + 5pqr^2 + 2pq - 2pqr^2 + \tfrac{1}{2}pq(6r - 6r^2 - 3pq)$.

$P_{AB} = r^2(1 - r)[(1 + r) + \tfrac{1}{2}(1 - r)] + 3pqr^2 + 2pq + 3pqr - 3pqr^2 - \tfrac{3}{2}p^2q^2$.

Therefore, $P_{AB} = \tfrac{1}{2}r^2(1 - r)(3 + r) + \tfrac{1}{2}pq(4 - 3pq + 6r)$. (7)

For comparison, the following formula, given by KOMATU, is offered:

$$P_{AB} = 2r^2(1 - r)(3 - r) + 2pq(2 + r - 4r^2) - \tfrac{3}{2}p^2q^2 + 14r^4\left[\left(\frac{p}{p + 2r}\right)^2 + \left(\frac{q}{q + 2r}\right)^2\right] - \frac{8pqr^4}{(p + 2r)(q + 2r)} - \frac{2pr^2}{p + 2r}[2r(4p + r) + q(p + 2q)] - \frac{2qr^2}{q + 2r}[2r(4q + r) + p(q + 2p)]. \quad (8)$$

The first thing that strikes one about KOMATU'S formula is that his final formula is far more complicated than the unsimplified sum given in equation (6) from which the formula (7) was derived. That the formula (8) is incorrect can be shown by setting $r = 0$, in which case the formula should reduce to equation (5) for the agglutinogens M and N, substituting the letters, m and n, for p and q, respectively. Whereas, according to equation (5), P_{AB} should equal $\tfrac{1}{2}pq(4-3pq)$ when $r=0$, on the other hand, from formula (8), P_{AB} reduces to $\tfrac{1}{2}pq(8-3pq)$. Since KOMATU himself asserts that equation (5) is correct, on this basis, formula (8) must be

invalid. On the other hand, formula (7) satisfies the requirement that it reduce the formula (5) when r = o. In a similar manner, the equation for P_{AB} should reduce to the formula for P_Q when either p or q = o, the letter r in the formula obtained in this manner corresponding to the letter v in the formula for P_Q, and $1-r$ corresponding to u. By inspection it will be found that when either p or q = o, formula (7) reduces to formula (1). On the other hand, KOMATU's formula (8) for P_{AB} does not reduce to his own formula for P_Q.

It is of interest to determine what contribution to the chance of identifying fraternal twins is made by the subgroups of A and AB. According to the theory of THOMSEN, FRIEDENREICH and WORSAAE (1930) the hereditary transmission of the groups, including the subgroups, is by means of four allelic genes, A_1, A_2, B and R where A_1, A_2 and B are dominant over R, and A_1 is dominant over A_2.

In table 4, we have summarized the contribution to the chance of recognizing fraternal twins of each mating listed there. Not every possible mating is tabulated; those which contribute nothing to the chances have been omitted in order to shorten the table. The value of $P_{A_1A_2B}$ is obtained by adding all the terms in the last column of the table. To simplify the resulting expression, the best procedure is to ascertain by how much $P_{A_1A_2B}$ exceeds P_{AB}, bearing in mind that $p_1+p_2=p$. The following expression is obtained:

$$P_{A_1A_2B} = P_{AB} + 4p_1p_2r^2 + 3p_1p_2qr + p_1^2p_2q + 2p_1p_2^2q + 3p_1p_2q^2 + 6p_1p_2^2r + \tfrac{3}{2}p_1^2p_2^2 + 3p_1^2p_2r + 2p_1p_2^3. \quad (9)$$

Simplifying, we obtain the following formula:

$$P_{A_1A_2B} = P_{AB} + p_1p_2(2p_2 + 3r + r^2 + p_1q + 3q^2 + p_2r - \tfrac{1}{2}p_1p_2). \quad (10)$$

Obviously, this formula reduces to P_{AB} when either p_1 or $p_2 = o$, as is to be expected.

It is of interest to apply formulas (7) and (10) to an actual case. As an example we can determine what the values of P_{AB} and $P_{A_1A_2B}$ are for the population studied by WIENER and ROTHBERG (1933). These investigators found the distribution of the groups and subgroups in a series of approximately 900 individuals in New York City to be:

$$O = 44.6\%,\ A_1 = 25.6\%,\ A_2 = 12.0\%,\ B = 13.6\%,\ A_1B = 3.1\% \text{ and } A_2B = 1.2\%.$$

Therefore, by applying the formulas for the frequency of the genes given by WIENER (1939) we have:

$$p_1 = 15.3\%,\ p_2 = 8.4\%,\ q = 9.3\% \text{ and } r = 66.8\%.$$

Substituting the values p = 0.237, q = .093 and r = 0.668 in formula (7),

TABLE 4

Chances of recognizing fraternal twins by the six groups, **O, A_1, A_2, B, A_1B** *and* **A_2B**.

MATING	FREQUENCY OF MATING	FREQUENCY OF CHILDREN OF GROUP						CHANCE OF NON-IDENTITY OF GROUPS OF FRATERNAL TWINS
		O	A_1	A_2	B	A_1B	A_2B	
1) $RR \times A_1A_2$	$4p_1p_2r^2$	0	50	50	0	0	0	$2p_1p_2r^2$
2) $RR \times A_1R$	$4p_1r^2$	50	50	0	0	0	0	$2p_1r^2$
3) $RR \times A_2R$	$4p_2r^2$	50	0	50	0	0	0	$2p_2r^2$
4) $RR \times BR$	$4qr^2$	50	0	0	50	0	0	$2qr^2$
5) $RR \times A_1B$	$4p_1qr^2$	0	50	0	50	0	0	$2p_1qr^2$
6) $RR \times A_2B$	$4p_2qr^2$	0	0	50	50	0	0	$2p_2qr^2$
7) $A_1A_1 \times BR$	$4p_1^2qr$	0	50	0	0	50	0	$2p_1^2qr$
8) $A_1A_1 \times A_1B$	$4p_1^2q$	0	50	0	0	50	0	$2p_1^2q$
9) $A_1A_1 \times A_2B$	$4p_1^2p_2q$	0	50	0	0	50	0	$2p_1^2p_2q$
10) $A_1A_2 \times A_1A_2$	$4p_1^2p_2^2$	0	75	25	0	0	0	$\frac{1}{2}p_1^2p_2^2$
11) $A_1A_2 \times A_1R$	$8p_1^2p_2r$	0	75	25	0	0	0	$3p_1^2p_2r$
12) $A_1A_2 \times A_2A_2$	$4p_1p_2^3$	0	50	50	0	0	0	$2p_1p_2^3$
13) $A_1A_2 \times A_2R$	$8p_1p_2^2r$	0	50	50	0	0	0	$4p_1p_2^2r$
14) $A_1A_2 \times BB$	$4p_1p_2q^2$	0	0	0	0	50	50	$2p_1p_2q^2$
15) $A_1A_2 \times BR$	$8p_1p_2qr$	0	25	25	0	25	25	$6p_1p_2qr$
16) $A_1A_2 \times A_1B$	$8p_1^2p_2q$	0	50	0	0	25	25	$5p_1^2p_2q$
17) $A_1A_2 \times A_2B$	$8p_1p_2^2q$	0	25	25	0	25	25	$6p_1p_2^2q$
18) $A_1R \times A_1R$	$4p_1^2r^2$	25	75	0	0	0	0	$\frac{1}{2}p_1^2r^2$
19) $A_1R \times A_2A_2$	$4p_1p_2^2r$	0	50	50	0	0	0	$2p_1p_2^2r$
20) $A_1R \times A_2R$	$8p_1p_2r^2$	25	50	25	0	0	0	$5p_1p_2r^2$
21) $A_1R \times BB$	$4p_1q^2r$	0	0	0	50	50	0	$2p_1q^2r$
22) $A_1R \times BR$	$8p_1qr^2$	25	25	0	25	25	0	$6p_1qr^2$
23) $A_1R \times A_1B$	$8p_1^2qr$	0	50	0	25	25	0	$5p_1^2qr$
24) $A_1R \times A_2B$	$8p_1p_2qr$	0	25	25	25	25	0	$6p_1p_2qr$
25) $A_2A_2 \times BR$	$4p_2^2qr$	0	0	50	0	0	50	$2p_2^2qr$
26) $A_2A_2 \times A_1B$	$4p_1p_2^2q$	0	50	0	0	0	50	$2p_1p_2^2q$
27) $A_2A_2 \times A_2B$	$4p_2^3q$	0	0	50	0	0	50	$2p_2^3q$
28) $A_2R \times A_2R$	$4p_2^2r_2$	25	0	75	0	0	0	$\frac{1}{2}p_2^2r^2$
29) $A_2R \times BB$	$4p_2q^2r$	0	0	0	50	0	50	$2p_2q^2r$
30) $A_2R \times BR$	$8p_2qr^2$	25	0	25	25	0	25	$6p_2qr^2$
31) $A_2R \times A_1B$	$8p_1p_2qr$	0	50	0	25	0	25	$5p_1p_2qr$
32) $A_2R \times A_2B$	$8p_2^2qr$	0	0	50	25	0	25	$5p_2^2qr$
33) $BB \times A_1B$	$4p_1q^3$	0	0	0	50	50	0	$2p_1q^3$
34) $BB \times A_2B$	$4p_2q^3$	0	0	0	50	0	50	$2p_2q^3$
35) $BR \times BR$	$4q^2r^2$	25	0	0	75	0	0	$\frac{1}{2}q^2r^2$
36) $BR \times A_1B$	$8p_1q^2r$	0	25	0	50	25	0	$5p_1q^2r$
37) $BR \times A_2B$	$8p_2q^2r$	0	0	25	50	0	25	$5p_2q^2r$
38) $A_1B \times A_1B$	$4p_1^2q^2$	0	25	0	25	50	0	$\frac{1}{2}p_1^2q^2$
39) $A_1B \times A_2B$	$8p_1p_2q^2$	0	25	0	25	25	25	$6p_1p_2q^2$
40) $A_2B \times A_2B$	$4p_2^2q^2$	0	0	25	25	0	50	$\frac{1}{2}p_2^2q^2$

we find that $P_{AB} = 35.9$ percent. From equation (10) and the fact that $p_1 = 15.3$ percent and $p_2 = 8.4$ percent, we find that $P_{A_1A_2B} = 0.359 + 0.035 = 39.4$ percent.

It is a simple matter now to calculate how frequently fraternal twins can be diagnosed if one takes into account the sex of the twins, the blood groups, subgroups and MN-types. From equation (3), we have:

$P = 1-(1-0.5)(1-0.394)(1-0.403) = 0.819$, taking the chance of diagnosing fraternal twins by means of sex equal to 0.5, $P_{A_1A_2B} = 0.394$ and $P_{MN} = 0.403$.

SUMMARY

Formulas have been derived for the chances of proving that a pair of fraternal twins are biovular (1) by means of characters transmitted as simple Mendelian dominants, (2) by the four blood groups, O, A, B and AB, (3) by the agglutinogens, M and N, and (4) by the subgroups of groups A and AB. The formulas reported by KOMATU were found to be different from those derived by the present writers. It was demonstrated that KOMATU's formulas must be incorrect since they contradict one another.

Using the formulas derived by the present writers, it was calculated that for a population such as the one in New York City, 81.9 percent of fraternal twins could be diagnosed as biovular by taking into account their sex, blood groups, subgroups and MN types.

ADDENDUM

As further evidence of the accuracy of our formulas (as well as the inaccuracy of KOMATU's formulas), we cite a paper by RIFE (1939) which was brought to our attention while the present communication was in press. RIFE has also calculated the frequency of discordance in fraternal twins in various simple types of heredity, though he does not give the general formulas. He finds in the case of a single pair of alleles, one of which is dominant, the maximum value of P_Q to be about 27.2 percent, which coincides precisely with our own results. His value for P_{MN} (about 40 percent) is the same as ours (40.3) and his value for P_{AB} (35 percent) also closely approximates our own (35.9).

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A COLORIMETRIC STUDY OF GENIC EFFECT ON GUINEA-PIG COAT COLOR

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INTRODUCTION

ONE approach to the study of gene physiology is obviously a description of the end-products of genic interaction. Estimates of quantity and an elucidation of any qualitative differences between or within allelic series should aid in the establishment of a general theory and thus in an understanding of the links between gene and phenotype. With respect to pigmentation in the guinea-pig, it has seemed desirable to continue the attack started by RUSSELL (1939) on the quantitative aspects of the problem although the need for qualitative chemical analysis cannot be denied since, at the present time, we have only rather meager suggestions as to differences in kind. We know, for example, that in general the yellow pigments from mammalian hair are more readily soluble in cold dilute alkalis than are the sepia melanins, although information on other differences between these pigments, and possible variations within the sepia and yellow end-products are still lacking. WRIGHT's work (1927) with the Milton-Bradley color-wheel led him to the suggestion that the differences between red and yellow grades were more than simple quantitative variations since the proportion of orange required to match red skins (using black, white, yellow and orange) was greater than that needed for the yellow ones. The more recent work of DANIEL (1938) giving spectrophotometric measurements of solutions of sepia pigment from the hair of the mouse has indicated, however, that qualitative differences probably do not exist within the genotypes tested (combinations of the A, B, C series). That care must be exercised in drawing conclusions from these data is indicated by the author's statement to the effect that there is still some uncertainty concerning the method.

Although the quantitative measurement of hair pigments is fraught with difficulties, some rather satisfactory attempts have recently been made by EINSELE (1937) and DUNN and EINSELE (1938) for the mouse, and by RUSSELL (1939) for the guinea-pig. In the latter case, advantage was taken of an observation made by DURHAM (1904) and others that yellow pigment is rather readily soluble in cold, dilute alkali. RUSSELL, accordingly, was able to develop a colorimetric method for the determination of intensity differences among animals with yellow fur. Since the sepia pigments are relatively resistant to this treatment the same method could

not be used for this color quality. She therefore developed another technique by which the pigment was first separated from a known weight of hair, essentially according to the method of EINSELE, and then titrated with $KMnO_4$ to determine the amount of this substance which could be reduced by the isolated pigment. EINSELE, meanwhile, had found that the dark pigments of mouse hair could be made to dissolve by boiling in solutions of KOH (1937). The present investigation was undertaken for the purpose of extending the observations on the yellow series and of developing a colorimetric method for the sepia genotypes. It was also hoped that if the sepia pigments could be made to go into solution, the resulting color quality might be sufficiently similar to that of yellow to warrant quantitative comparisons between the two series. A colorimetric method for the sepia series has been developed, but the solutions were found to have a somewhat duskier hue than those of the yellow series. Thus comparisons between the two qualities are not available.

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MATERIALS AND METHODS

The major color-factors of the guinea-pig have been so thoroughly investigated by WRIGHT (1915, 1916, 1917, 1923, 1925, 1927) that it is hardly necessary to describe the stocks at length. Of the seven major series of alleles, readings were made on compounds of the members of four. These include E, e in which E governs the production of sepia, and e of yellow quality; the albino series C, c^k, c^d, c^r, c^a the members of which modify the intensity of both yellow and sepia; the P, p series in which the homozygous recessive dilutes sepia but has no effect on yellow; the F, f series in which ff reduces yellow but has no effect on sepia except in combinations involving $E-ffpp$ in which case the animals are of a low grade of yellow or white. Practical considerations have made it impossible up to the present time to introduce the various combinations into a single isogenic stock although the desirability of this is evident.

The routine procedure in the color experiments was to grade each animal within a day or so after birth by means of two qualitatively different sets of pelts standardized according to graduated steps of intensity of yellow and sepia, respectively. Genotypes were assigned on the basis of color quality and intensity, the genotypes of the parents, and in some cases, breeding tests. Three to four weeks after birth, hair was clipped to approximately equal length of base from the mid-dorsal region of the body and stored in manila envelopes away from any intense source of light. The time between collection and use varied from a few weeks to a year.

The yellow series

The technique for the yellow genotypes was substantially that followed by RUSSELL. The hair was first washed in two changes of cold ether and dried. Samples weighing 100 mgs (weighed to the nearest mg) were then placed in 10 cc of a .5N solution of NaOH and allowed to stand for five days. The unhydrolyzed hair was removed by filtration and the resulting colored solutions were compared to each other in a colorimeter (Klett biometer). As explained by RUSSELL, the intensity of color seen in the field of a colorimeter depends upon the intensity of the colored solution and the height of the column through which the light passes. Furthermore, if two intensities are matched, intensity 1 : intensity 2 = height 2 : height 1. It follows from this relationship that if height 1 is fixed arbitrarily (for example, at 15 mm) and height 2 is determined by matching the intensity of solution 2 against that of solution 1, the ratio of these two heights will give a measure of the relative intensities of the two solutions.

Before a given series of comparisons was made, the colorimeter was calibrated by placing a portion of a given standard in each of the two colorimeter cups. These identical samples were read against each other in the manner described above. The colorimeter light was adjusted until it was possible to secure five consecutive readings in which height 2 fell within a range of $15 \pm .4$ mm (height $1 = 15$ mm).

The determinations for a given animal were made as follows. The duplicate experimental solutions from a particular sample were first compared to each other by means of ten consecutive, independent readings. Then each was compared with one of a series of standards made by diluting an intense solution from the hair of a single yellow adult guinea-pig (grade 9 and constitution $eeC-F-$) with the .5N solution of NaOH in the proportions of 1:0, 2:1, 1:1, 1:2, 1:6. Thus for a given animal there were two comparisons with standard, each involving ten readings, hence ten ratios ($h1/h2$). The arithmetic average of the means of these two series of ratios was taken as the colorimetric value (CV) for the animal.

The only departure from the above procedure was in the case of grades 0 and 1, at the lower end of the intensity scale. Solutions from these grades were so light that the two sides of the colorimeter field were indistinguishable within a mean range of about 4 mm. The preparations were, however, not colorless but of a distinctly yellow tinge and it was therefore desirable to have at least an approximate value for them on the colorimetric scale. The practice was, accordingly, to use the lightest standard and with that to make four estimates each of the upper and lower limits of the indistinguishable band. The mid-point of this range was taken as the final determination.

The sepia series

The method for separating the sepia pigment from the keratin structure was essentially that developed by EINSELE (1937) and later used by RUSSELL. The keratin was hydrolyzed in 6N HCl and the resulting suspensions containing the pigment granules, were centrifuged and washed in water to separate the hydrolysate from the pigment (plus the small quantities of occluded impurities). For every animal in the present work two samples, each weighing 100 mgs, were treated in this manner. Considerable difficulty in separation was experienced with grades 10–12 but by prolonged and repeated centrifugation and careful slowing of the centrifuge, success was finally attained in four cases. These experiences agree in general with those of RUSSELL who circumvented the difficulty with centrifugation by adding weighed quantities of the coarsely granular pigment from black animals, to the suspensions, and was thus able to clear the hydrolysate, since the lighter pigments were precipitated by occlusion. The author did not use this method for the following reason. The data secured by RUSSELL on the percentage weight of melanin in sepia hair indicated that the proportion of pigment in these genotypes is very small (a mean of 1.37 percent for the genotype $E-c^dc^aP-$). Thus for the lighter grades reliable weights could not be attained. The experimental error in adding extra black pigment for purposes of centrifugation might, therefore, tend to obscure the true values for the pale genotypes. This may account to some extent for the fluctuating permanganate values at the lower end of the scale.

A different procedure from that used by RUSSELL was followed after the technique of separation had been completed. The pigment (two samples from each animal) was put into solution by boiling for $1\frac{3}{4}$ to $2\frac{1}{2}$ hours in a reflux condenser with a .2N solution of KOH. The quantity of alkali was varied from 25 cc to 100 cc in accordance with the amount of pigment judged to be present on the basis of grade. This procedure was followed in order that all solutions might be of an intensity suitable for reading against a standard made by dissolving the pigment in 100 mgs of hair from an animal of grade 19 and constitution $E-c^rc^rP-$, in 100 cc of the solution of KOH. The alkali was added directly to the pigment plus the small amount of water left after the last decantation. Consequent slight variation in pH could have had little if any effect on the readings since it will be evident from a later analysis that the errors between two samples from the same animal were small.

Preliminary work indicated that it was not always easy to tell exactly at what point the pigment went into solution. At times the "solution" would appear to be clear, yet filtration and centrifugation indicated that small particles were still in suspension. The practice, therefore, was to boil the suspensions for about one-half hour beyond the time when, to the naked

eye, they looked clear. In no case did subsequent filtration or centrifugation indicate any detectable, undissolved granules. Tests of additional boiling were made on 14 of these solutions. In each case, two samples of sufficient quantity to fill the colorimeter cups were read against each other. One of these was then saved and all of the remaining solution was boiled for an additional hour. Determinations for the portion which had undergone extra boiling were made against the sample which had been saved. An analysis by Student's method for paired comparisons yielded a value of 1.24 for t. This shows that no significant change in intensity had occurred. It is assumed, therefore, that the technique of boiling beyond the time when the suspensions appeared to have gone into solution had no appreciable effect, at least within the limits indicated.

After the solutions had been adjusted for volume, they were read against the standard ($E-c^{r}c^{r}P-$, grade 19) and against each other. As in the yellows, each determination was taken as the mean of ten ratios secured from ten independent readings. The colorimeter value (CV) for a given animal is the arithmetic average of the two means secured from reading the experimental solutions against standard, except for the lower grades in which less than 100 cc of KOH was used. In these cases the average was multiplied by the appropriate factor, to give the true colorimetric value.

The possibility that changes in standard might have occurred was checked at the end of four months (three weeks after the last experimental determinations were made) by preparing two solutions from the same sample of hair which had been used in the preparation of the standard itself. These two solutions when compared with the original standard gave mean readings of 1.076 and 1.063. Such figures indicate that the standard probably had faded slightly in spite of the ordinary precaution of keeping it tightly stoppered in a photographic dark room, except when readings were being made.

A second test for the possible effects of change in standard on the readings was made by examining the determinations within grades 21, 20, 19, 18 (groups having the largest numbers) for trend. The data for each group were trichotomized according to the time when the experiments were performed, and a grand unweighted mean for each of the three periods was determined. These values were 1.137, 1.157, 1.189 for the first, second and third periods, respectively. The numbers are too small for an elaborate statistical study of trend, but the determinations for the three periods are at least consistent with the view that the standard did undergo a slight reduction in intensity. Such a change is not regarded as serious since it must have been small and it will be evident that the variability within both grade and genotype is relatively large.

RESULTS AND DISCUSSION OF RESULTS

Errors of the methods

Some measure of the reliability of the methods is necessary. The errors can be grouped into two classes: (1) the experimental error involved in sampling from the hair, weighing the hair and preparing the solutions for colorimetry, (2) the colorimetric error. The latter was determined directly, in the case of the readings of sample two against sample one, by finding the average variance within the sets of ten readings ($\sum_1^n \sum_1^{10}(v-m)^2/9n$) where n is the number of comparisons, the v's are the individual readings and the m's are the means of the sets of ten readings. In the case of yellow (101 cases, including all of grades 2–12 and two of grade one) this variance was .00090, indicating a standard deviation of .030. The standard error of the mean of ten readings was thus only .0095 ($=.030/\sqrt{10}$) or 0.95 percent, since the mean of the 101 means was 1.00.

The variance of set means (sample two read directly against sample one) was .00295 ($=\Sigma_1^{101}[m-1.00]^2/_{100}$). This is compounded of the experimental errors in the preparation of both samples ($2\sigma_e^2$) and the colorimetric error (σ_c^2) which we have found to be .000090 ($=.0095^2$). The colorimetric error is thus only about three percent of the total error in this case.

In comparisons of sample one with standard, experimental error is involved only once. Thus the colorimetric error should constitute nearly twice as great a proportion of the total as above, viz., $\sigma_c^2/\sigma_e^2+\sigma_c^2$, or 5.9 percent, provided the colorimetric error is the same (on the appropriate scale) as in the comparison with the other sample.

A second test can be obtained from the correlation of the mean direct readings of one sample against the other with the corresponding ratio of the mean readings of each against the standard. If there were no colorimetric error, the correlation (r) would necessarily be perfect. The actual value of r in the yellow series was $.89 \pm .02$. The portion of the total error due to colorimetry can be found as follows. A direct reading of one sample against the other, deviates in a particular case because of the errors in the preparation of both solutions and also because of *one* error of colorimetry. The ratio of the mean readings against standard deviates from one because of the same errors in preparation plus *two* errors of colorimetry. Let $\sigma_{c_1}^2$ equal the squared standard error of colorimetry in this case. It may differ from σ_c^2 since the samples may vary considerably from standard in concentration. The degree of determination by errors of preparation is

$\frac{2\sigma_e^2}{2\sigma_e^2+\sigma_c^2}=.97$ in the former case and $\frac{2\sigma_e^2}{2\sigma_e^2+2\sigma_{c_1}^2}$ in the latter. The correlation coefficient may be equated to the product of the square roots of

these expressions which are the path coefficients measuring the contributions of the common factors. This yields $2\sigma_e^2/2\sigma_e^2+2\sigma_{e_1}^2=.82$. The portion of the variance due to experimental error in a comparison of one sample with standard should agree $(\sigma_e^2/\sigma_e^2+\sigma_{e_1}^2)$ giving 18 percent as the portion of the variance in this case due to colorimetry. This is about three times as large as the estimate derived from the readings of the samples against each other and seems to indicate that errors of colorimetry are much greater in comparisons with standard. Nevertheless the colorimetric error is still a minor portion of the total error. The experimental error in preparing one sample may be estimated as .00144 ($\frac{1}{2}$·.00296–.00009). Since $\sigma_e^2/\sigma_e^2+\sigma_{e_1}^2=.82$, the total error in grading one sample with the standard $(\sigma_e^2+\sigma_{e_1}^2)$ may be estimated as $.00176\left(=\frac{.00144}{.82}\right)$. The standard deviation of total errors for one sample is thus about 4.2 percent $(=\sqrt{.00176})$. The standard deviation for the average of two samples is thus about 3.0 percent $\left(=\frac{4.2}{\sqrt{2}}\right)$.

A comparable statistical analysis for the sepia genotypes (90 experiments run in duplicate and including grades 10–21) yielded a total variance of .00129 for the means of sets of direct readings of one sample against the other. The variance within the sets of ten readings was .00065 and thus the variance of means, due to colorimetry, was .000065 or about 5.0 percent of the total. This would imply about 9.5 percent determination of the total squared error by colorimetric errors in the case of the reading of one sample against standard, if the colorimetric errors remain the same. As before, however, analysis of the correlation between the direct readings, sample two against sample one, and the ratio of the readings against standard, indicates a larger average colorimetric error in the readings against standard. The correlation coefficient was $.86\pm.03$ in this case. This gives 78 percent $\left(=\frac{.86^2}{.95}\right)$ as the estimated portion of the variance due to experimental errors, in comparisons with standard, and 22 percent (instead of 9.5 percent) as the portion due to colorimetric errors. The total error in a comparison of one sample with standard may be estimated as $.00079\left(=\frac{.000618}{.78}\right)$. The standard deviation of total errors for one sample is thus about 2.8 percent $(=\sqrt{.00079})$ and the standard deviation for the average of two samples is about 2.0 percent. It should be pointed out that

standard deviations of such a size are a little low for the method as a whole. This is accounted for by the following considerations. All experiments in which the two samples from the same animal were grossly different were repeated. In eight of the nine cases in which this procedure was followed the solutions of the second gave readings within a ten percent range of each other and were, therefore, regarded as reflecting a more accurate value for the animal under experimentation. The most probable source of gross error in such cases was loss of pigment during decantation of the hydrolysate or wash water.

The yellow series

The colorimetric values for the yellow series are given by grade in table 1 and by genotype in table 2 and figure 1. The standard errors in tables 1 and 2 were calculated in the usual manner but from a generalized standard deviation secured by multiplying the mean for each grade or genotype, as the case might be, by the weighted mean coefficient of variability for all grades or genotypes (weight = n − 1). This procedure was followed in order to find a reasonably reliable measure of variability for groups in which the numbers were so small that standard errors calculated from the actual standard deviations would have little meaning. In grades 10, 7 and 4, classes which occur most frequently, and which, therefore, may be assumed to show the most reliable means, the generalized standard deviation varies only slightly from that calculated directly. The values are reduced in

TABLE 1

A comparison of the colorimetric values for grades of yellow with those estimated by Russell.

GRADE	PRESENT DATA					RUSSELL'S DATA		
	NO. OF ANIMALS	MEAN CV	σ	GENERALIZED σ	S.E.	NO. OF ANIMALS	MEAN CV	GENERALIZED S.E.
12	3	1.849	.156	.348	±.201	1	2.654	±.667
11	6	1.516	.258	.285	.117	9	3.020	.253
10	13	1.460	.276	.275	.076	15	2.872	.186
9	6	1.164	.167	.219	.090	2	2.237	.397
8	5	0.848	.104	.160	.071	6	1.403	.144
7	27	0.618	.101	.116	.022	34	1.039	.045
6	10	0.544	.112	.102	.032	12	0.778	.056
5	7	0.413	.104	.078	.029	17	0.627	.038
4	13	0.235	.054	.044	.012	22	0.552	.030
3	7	0.173	.054	.035	.013	12	0.357	.026
2	2	0.133	.019	.025	.018	4	0.239	.030
1	3	0.064	.015	.020	.012	1	0.122	.031
0	4	0.057	.005	.017	±.008	3	0.120	±.017

grades 3–6 but in all other instances they are about the same or larger than the conventional standard deviations.

The uncertainty of the means for very high grades (12, 11, 10) is probably partly due to the fact that animals of high intensity do not hold their original color as do those of lower grades (exclusive of *f f* genotypes). After the data on grades 12 and 11 had been secured, the small samples of hair

TABLE 2

A comparison of the colorimetric values of yellow genotypes with those estimated by Russell.

	PRESENT DATA						RUSSELL'S DATA			
	eeF–						*eeF–*			
C SERIES	NO. OF ANIMALS	MEAN GRADE	MEAN CV	σ	GENERALIZED σ	S.E.	NO. OF ANIMALS	MEAN CV	S.E.	RUSSELL'S TRANSFORMED CV
Cc^k	2	11.5	1.643	.545	.360	±.255	..	.....	±....	.....
Cc^d	9	10.0	1.422	.273	.312	.104	..	.....	....	.....
Cc^r	3	9.3	1.225	.208	.269	.155	..	.....	....	.....
Cc^a	6	10.3	1.530	.301	.335	.137	..	.....	....	.....
C–*	28	10.2	1.450	.300	.318	.060	27	2.866	.114	1.571
c^kc^k	8	6.8	.502	.069	.110	.039	10	.949	.055	.474
c^kc^d	1	7.0	.611	....	.134	.134	6	1.031	.150	.521
c^kc^r	1	4.0	.183	....	.040	.040	13	.616	.048	.284
c^kc^a	4	4.0	.513	.039	.047	.023	4	.489	.025	.211
c^dc^d	21	6.8	.676	.129	.148	.032	23	1.150	.082	.589
c^dc^r	10	4.6	.365	.118	.080	.025	19	.593	.046	.271
c^dc^a	1	4.0	.279	....	.061	.061	18	.484	.036	.208
c^rc^r	1	0.0	.054	....	.012	.012	2	.133	.017	.007
c^rc^a	2	0.0	.058	....	.009	.009	..	.....	....	.....
c^ac^a	1	0.0	.058	....	.013	.013	..	.....	....	.....
	eeff						*eeff*			
C–	11	6.8	.636	.138	.139	.042	7	.888	.053	.439
c^kc^k	2	1.0	.057	.011	.012	.009	..	.....	....	.....
c^dc^d	10	2.7	.150	.051	.033	±.010	10	.280	±.031	.092

* Total includes genotypes in which the second allele was unknown.

left from the experiments were examined and compared with the standard pelts. Although it is difficult to evaluate small wisps of hair, there was no doubt whatever that all of the samples of grade 12 and three of grade 11 had faded noticeably. These observations, therefore, indicate that the lighter intensities of adult animals are foreshadowed in some cases, at least, by a fading of the color as early as three to four weeks of age.

It will be noted that in harmony with the previous observations of

RUSSELL, the steps from 0–12 can be regarded approximately as percentage rather than absolute increments. A transformation comparable to hers was therefore made by plotting the logarithms of the corrected colorimetric means (experimental determinations minus .057, the value for white) against the corresponding grades and passing a line through the points by the method of least squares. The weights (W) were inversely

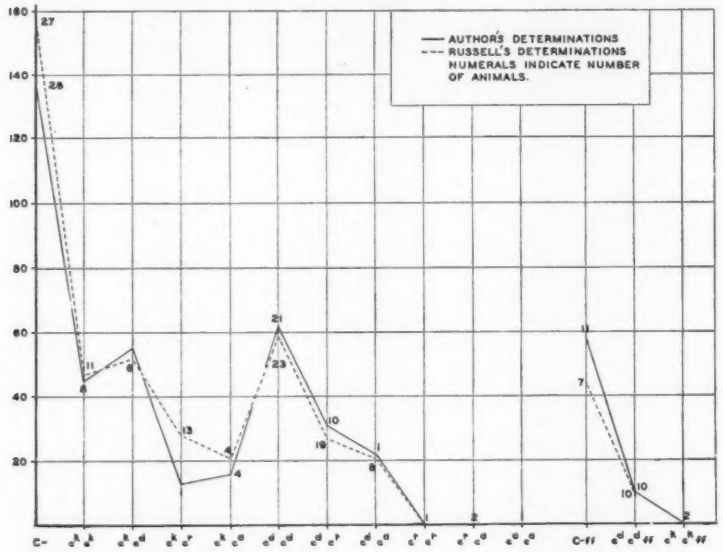

FIGURE 1.—A comparison of the corrected colorimetric values for yellow genotypes with those estimated by RUSSELL. Scales are made roughly comparable by multiplying RUSSELL'S corrected values by .572.

proportional to the estimated squared standard errors on the logarithmic scale. Since small deviations (δCV) on the original scale become $\frac{\delta CV \log_{10} e}{CV - .057}$ on the logarithmic scale, the standard error on the latter was taken as $\frac{.434\,SE}{CV - .057}$. The standard error of the slope $\frac{\Sigma WGY - \bar{Y}\Sigma WG}{\Sigma WG^2 - \bar{G}\Sigma WG}$ was taken as $\frac{.434}{\sqrt{\Sigma WG^2 - \bar{G}\Sigma WG}}$. The close agreement between the author's results and those of RUSSELL is revealed by the essential identity of the slopes in the following equations

$$\log (CV-.057) = \bar{8}.735+.138G \text{ (not weighted)}$$

$$\log (CV-.057) = \bar{8}.812+.132G\pm.0039 \text{ (weighted)}$$

and those quoted by RUSSELL

$$\log (CV-.12) = \bar{8}.987+.136G \text{ (not weighted)}$$

$$\log (CV-.12) = \bar{9}.032+.135G\pm.0046 \text{ (weighted).}$$

If RUSSELL's line be made to intersect the author's at grade 7, her equation becomes

$$\log (CV-.12) = \bar{8}.779+.135G$$

It is obvious that neither weighting nor the use of a series of standards in place of the single one of grade 7 used by Russell has resulted in any substantial differences. Slightly significant departures from linearity (weighted line) are indicated by t values of -2.9, -3.1, $+3.0$, -2.7, -2.8 for grades 3, 4, 6, 11, 12 respectively, and also by grouping of the signs for t in grades 1–4, 5–10, 11–12.

The tabulations according to genotype (table 2 and figure 1) are of interest in evaluating the dominance relations of the *C* series. In order to make the scales of RUSSELL and the author comparable, the corrected values as given by her must be multiplied by .572, the factor which was found to make her value for grade 7 as given by the line coincide with that of the author. Table 3 gives the percentage of the intensity of type

TABLE 3

Percentage of the intensity of C—F— in other gene combinations.

GENOTYPE	PRESENT DATA		RUSSELL'S DATA	
	NO. OF ANIMALS	PERCENT	NO. OF ANIMALS	PERCENT
$C-F-$	28	100.0	27	100.0
c^bc^bF-	8	32.0	11	30.2
c^bc^dF-	1	39.8	6	33.2
c^bc^rF-	1	9.0	13	18.1
c^bc^aF-	4	11.2	4	13.6
c^dc^dF-	21	44.4	23	37.5
c^dc^rF-	10	22.2	19	17.3
c^dc^aF-	1	15.9	8	13.2
$c^{ra}c^{ra}F-$*	4	0.0	2	0.0
$C-ff$	11	41.6	7	27.9
c^bc^bff	2	0.0	0	.....
c^dc^dff	10	6.7	10	5.9

* The double superscripts used throughout this paper signify all of the possible gene combinations indicated by the letters. For example, $eec^{ra}c^{ra}F-=eec^rc^rF-$, eec^rc^aF-, eec^ac^aF-, all phenotypically white; $eec^dc^{ra}ff=eec^dc^rff$, eec^dc^aff, phenotypically white; $eec^dc^{ra}F-=eec^dc^rF-$, eec^dc^aF-, phenotypically cream.

($C-F-$) found in other combinations (after subtraction of the correction factor .057).

The author's determinations are in essential agreement with the previous observations of RUSSELL and also with expectations based on WRIGHT'S grades. The evidence indicates that C is dominant over its lower alleles, that $c^k\,c^k$ and $c^d\,c^d$ reduce the intensity markedly (to 32 and 44 percent of $C-F-$, respectively, after subtraction of the correction factor .057), that with $c^r\,c^r$ and $c^a\,c^a$ the threshold for the production of yellow has not been attained. The readings for $c^k\,c^k$, $c^k\,c^d$, $c^d\,c^d$ agree with all previous

TABLE 4

A comparison of the colorimetric and permanganate numbers (PN) for sepias tabulated by grade.

	PRESENT DATA					RUSSELL'S DATA		
GRADE	NO. OF ANIMALS	MEAN CV	σ	GENERALIZED σ	S.E.	NO. OF ANIMALS	MEAN* PN	S.E.
21	18	1.440	.212	.214	±.050	9	144	± 7
20	13	1.297	.176	.192	.053	5	115	7
19	17	1.011	.159	.150	.036	6	120	7
18	10	.894	.134	.133	.042	1	103	15
17	9	.755	.163	.112	.037	2	96	10
16	4	.782	.066	.116	.058	1	99	15
15	7	.609	.059	.090	.034	6	75	5
14	6	.526	.101	.078	.032	2	64	6
13	2	.478	.021	.071	.051	3	56	6
12	1	.347	....	.052	.052	2	62	7
11	2	.330	.039	.049	.035	..	...	..
10	1	.390	....	.058	±.058	..	...	±..

* PN is the abbreviation for "permanganate number" as defined by RUSSELL

$$(PN) = cc\ KMnO_4 \times N KMnO_4 \times 100/\text{grams hair.}$$

The values given in the table are uncorrected. To secure the corrected mean in each case subtract 12, the estimated value for adsorbed impurities.

results in falling within a relatively narrow range (weighted averages: present data: 40.9 percent of $C-F-$, RUSSELL'S data 34.8 percent) There are, however, differences: $c^d\,c^d$ is significantly more intense than $c^k\,c^k$ ($t=4.7$). Similarly $c^k\,c^r$, $c^k\,c^a$, $c^d\,c^r$, $c^d\,c^a$ fall within narrow limits, but at a level somewhat less than half that of the preceding genotypes (weighted average: present data 45.3 percent of $c^{kd}\,c^{kd}$, RUSSELL'S data 47.1 percent). Again the combinations involving c^d are significantly more intense than those involving c^k ($t=4.0$). The apparently greater intensity of c^k combinations is contrary to the slightly lower intensities observed by WRIGHT in the stocks on which he published in 1927. Further study will be necessary to determine how far these differences are due to real variations in

the effects of the genes and how far to differences in associated minor factors.

The drastic effect of ff is revealed by readings from $ee\,C-F-$, $ee\,C-ff$; $ee\,c^d c^d\,F-$, $ee\,c^d\,c^d\,ff$. In the presence of C, ff has about 42 percent (present data) or 28 percent (RUSSELL'S data) as much pigment as F. In the presence of $c^d\,c^d$, ff has about 15 percent (present data) or 16 percent

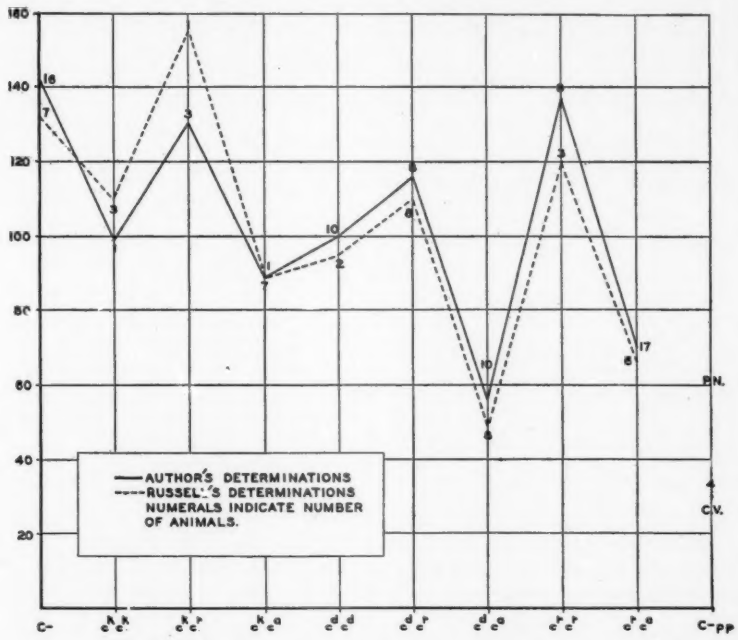

FIGURE 2.—A comparison of the colorimetric values and permanganate numbers for sepia genotypes. Scales are made comparable by multiplying the corrected permanganate numbers by .0101.

(RUSSELL'S data) as much pigment as F. The disproportionately larger effect in $c^d\,c^d$ foreshadows the complete elimination of yellow in $c^d\,c^{ra}\,ff$ in contrast with the considerable quantity present in $c^d\,c^{ra}\,F-$.

The sepia series

The data for the sepia series are given by grade in table 4 and for genotype in table 5 and figure 2. The same procedure was followed in calculating the standard errors as that previously described for the yellow series. Since there are probably real differences in the coefficients of variability of genotypes (for example, $c^r\,c^a$ extending over 7–10 grades in comparison

to c^k c^{ka} which cover only four grades, WRIGHT 1925) it is probably not as legitimate to generalize the standard deviations of genotypes as grades. In all cases standard deviations calculated in the usual manner are included together with the generalized standard deviations.

The determinations by grade show a consistent drop from grade to grade except for 16 and 10 in which the numbers were small. As with the grades

TABLE 5

A comparison of the colorimetric and permanganate number (PN) for sepias tabulated by genotype.

	PRESENT DATA						RUSSELL'S DATA			
	E−P− DARK-EYED SEPIAS						*E−P−* DARK-EYED SEPIAS			
C SERIES	NO. OF ANIMALS	MEAN GRADE	MEAN CV	σ	GENERALIZED	S.E.	NO. OF ANIMALS	MEAN* PN	S.E.	TRANSFORMED PN
CC	1	19.00	1.329	....	.233	±.233	..	...	±..	.....
Cc^d	5	20.80	1.358	.221	.238	.076	..	...	..	.....
Cc^a	10	21.00	1.561	.171	.274	.123	..	...	..	.....
C−	16	20.75	1.420	.216	.249	.062	7	143	9	1.323
c^kc^k	1	21.00	.994	....	.174	.174	3	120	12	1.091
c^kc^r	3	20.00	1.297	.250	.228	.131	1	166	26	1.555
c^kc^a	7	18.86	.885	.098	.155	.059	1	100	17	.889
c^dc^d	10	18.40	.996	.160	.175	.055	2	106	13	.949
c^dc^r	8	19.25	1.164	.284	.204	.072	8	121	7	1.101
c^dc^a	10	14.20	.562	.106	.099	.060	8	60	4	.485
c^rc^r	8	20.00	1.368	.194	.240	.084	3	131	12	1.202
c^rc^a	17	16.18	.699	.156	.123	.030	6	77	6	.657
	E−pp PINK-EYED SEPIAS						*E−pp* PINK-EYED SEPIAS			
C−	4	11.00	.349	.036	.060	±.030	1	76	±14	.646

* The values given are uncorrected. To secure the corrected mean in each case subtract 12, the estimated value for adsorbed impurities.

of yellow, it is also obvious here that the absolute decrements are large at the upper end of the scale and comparatively small at the lower end. The same methods were therefore applied in calculating the line which best fits the points determined by the logarithms of the colorimetric values plotted against grade (G). The slopes found for the colorimetric values and RUSSELL'S titrations are shown in the following equations:

$\log CV = \bar{8}.893 + .060G$ (unweighted, grades 21–13)
$\log CV = \bar{8}.849 + .062G \pm .0030$ (weighted, grades 21–13)
$\log CV = \bar{8}.865 + .061G \pm .0025$ (weighted, grades 21–10)
$\log (PN - 12) = .997 + .053G \pm .0048$ (weighted, grades 21–13)
$\log (PN - 12) = .953 + .056G \pm .0025$ (weighted, grades 21–2).

The line given by RUSSELL'S weighted equation for grades 21–13 was transformed so as to intersect the author's line at grade 17. RUSSELL'S line then became:

$$\log (PN - .12) = \bar{9}.002 + .053G.$$

Evidently the use of weights makes substantially no difference to the slopes in the first two equations. RUSSELL'S data contain no determinations for grades 10 and 11 and only two for grade 12. Thus the most suitable equations for comparison are those for grades 21–13, although the

TABLE 6

Percentage of the intensity of C—P— in other gene combinations.

GENOTYPE	PRESENT DATA		RUSSELL'S DATA	
	NO. OF ANIMALS	PERCENT	NO. OF ANIMALS	PERCENT
$C-P-$	16	100.0	7	100.0
$c^k c^k P-$	1	70.0	3	82.5
$c^k c^r P-$	3	91.3	1	117.5
$c^k c^a P-$	7	62.3	1	67.2
$c^d c^d P-$	10	70.1	2	71.7
$c^d c^r P-$	8	82.0	8	83.2
$c^d c^a P-$	10	39.6	8	36.7
$c^r c^r P-$	8	96.3	3	90.9
$c^r c^a P-$	17	49.2	6	49.6
$C-pp$	4	24.6	1	49.7

line for the colorimetric values is sufficiently stable so that inclusion of grades 10, 11, 12 does not alter the slope appreciably. The difference in slope constants .062 and .053 for grades 21–13 as given by the colorimetric and permanganate methods cannot be regarded as significant ($t = 1.6$). The colorimetric equation shows no significant departure from linearity since a comparison of calculated and observed values gives estimates of 2.4 or less for t and the signs are not clustered. There is, of course, no equation which includes colorimetric readings for grades 2–12 to compare with RUSSELL'S in as much as the colorimetric method was not applicable to grades 2–9.

The close agreement between the permanganate and colorimetric values, especially in genotypes in which the numbers were fairly large, is shown in tables 5 and 6 and figure 2. The last column in table 5 gives the values of RUSSELL, transformed by multiplying each of her entries (corrected) by .0101, the factor found to make the lines of RUSSELL and the author coincide at grade 17. From tables 4, 5 and 6 and figure 2 we see evidence for the complete dominance of C over its lower alleles, a rather high degree of dominance of c^k over c^a ($c^k c^a = .89 c^k c^k$) and approximate intermediacy of $c^d c^a$ and $c^r c^a$ compared to $c^d c^d$ and $c^r c^r$ ($c^d c^a = .56\ c^d c^d$, $c^r c^a = .51\ c^r c^r$).

Determinations for $c^d\ c^r$ are elevated when compared to $c^d\ c^d$ as are also $c^k\ c^r$ when compared to $c^k\ c^k$, $c^r\ c^r$ compared to $c^d\ c^d$, and $c^r\ c^a$ compared to $c^d\ c^a$ (within the limits indicated by the standard errors). These data are confirmatory of previous observations made by WRIGHT on the basis of grade alone and also of RUSSELL'S results. The most serious discrepancy between the permanganate and colorimetric values is in the pink-eyed sepias of constitution $C-$ in which the ratios of $C-P-$ to $C-pp$ are approximately 2 and 4 respectively. The numbers are small, one and four, but the colorimetric value would appear to give the more reliable estimate as judged by the relatively small standard deviation and the appearance of the hair as determined by the grades assigned.

SOURCES OF VARIABILITY

The data indicate a rather wide variability within both grade and genotype. At first sight one might expect such fluctuations within genotype on the basis of a consideration of the range of grade within genotypes (WRIGHT 1927), but comparatively small differences within grades since all animals of a given grade look alike. A careful examination of the hair samples, however, indicates differences in the distribution of pigment from tip to base within grade. These differences are less developed in the short hair at birth (when the grades were assigned) than at three to four weeks. No attempt has been made to take the basal color into account in grading. Causes for this variation in gradient are as yet unanalyzed although there is evidence that modifying genetic factors play a role. In addition to this source of variability, subjective errors of grading are occasionally responsible for differences of a grade.

The reasons for the instability of readings within genotype are not wholly known, but some suggestions as to the sources of variation can be given. The non-isogeneity of the hereditary background certainly suggests one possible factor; the role of environmental agents is also of probable importance. There is no doubt about the presence of at least one modifying genetic factor which reduces the intensity of both yellow and sepia (unpublished data from a stock not included in the present investigation but derived from the lighter sepias and yellows of the stock used). The large amount of literature on temperature and plucking with its consequent cooling effect shows that the deposit of pigment may be modified by these factors. The fact that the standard pelts undergo some fading of color with time and exposure to light while being used points to light as another controlling source of variability. Competition for food within the cages may also help to account for differences within litter-mates of the same genetic constitution. Here it is appropriate to note the works of HARTWELL (1923) and HAYDAK (1935) which have shown that the intensity of the color in

black rats could be reduced by restrictions of diet. The intensity could be restored in part at least by resumption of the stock diets or others containing adequate sources of proteins such as tyrosine and tryptophane, and other food constituents. A consideration of all of the above factors leaves little wonder that the colorimetric and permanganate readings show comparatively large fluctuations.

SUMMARY

1. Colorimetric determinations of the amount of yellow pigment in the hair of known genotypes of guinea-pig have confirmed and extended the previous evaluations by RUSSELL.

2. A colorimetric method was developed by which it has been possible to secure measurements of sepia solutions for grades 10–21. These include black-eyed sepias and pink-eyed sepias of constitution $C-$. Determinations by this method closely parallel the findings of RUSSELL who used a very different method. (Titration of pigment with potassium permanganate.)

3. An analysis has shown that the total experimental error for the yellow series (one set of ten readings) was 4.2 percent and for the sepia series 2.8 percent. About 18 percent of the total error was due to colorimetry in the case of the yellow series, and 22 percent in the sepia series.

4. The colorimetric determinations indicate that C is completely dominant over its lower alleles in both the yellow and sepia series, and that $c^k c^a$, $c^k c^r$; $c^d c^a$, $c^d c^r$ compounds in the yellow series show somewhat less than half as much pigment as $c^k c^k$ and $c^d c^d$ respectively. Genotypes $c^r c^r$, $c^r c^a$, $c^a c^a$ are pure white in place of yellow. The drastic reduction of yellow by $f f$ is shown to be disproportionately larger in $c^d c^d$ than in $C-$. In the sepia series, c^k shows a fairly high degree of dominance over c^a, but little or no dominance is exhibited by c^d and c^r over $c^a(c^a c^a$, white). All comparisons show that c^r produces more sepia than c^d. Genotypes of $C-P-$ constitution gave readings which were roughly four times those of $C-p p$.

5. The determinations for grade and genotype in both series showed considerable variability. Suggestions as to factors which may control these fluctuations are given.

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ON THE IDENTIFICATION OF SEGREGATED PHENOTYPES IN PROGENY FROM CREEPER FOWL MATINGS

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IN 1930 LANDAUER and DUNN established that the genetic basis of the Creeper character in fowl is a single Mendelian factor pair, lethal in homozygous condition. The heterozygotes are viable and show the characteristic chondrodystrophy that has earned the designation 'Creeper.' The homozygotes as a rule show marked pathological changes on the third day of incubation and die shortly thereafter; occasionally they survive until near hatching time, in which case they are strikingly phokomelic (LANDAUER 1933). The present study has two general purposes: 1) to examine the time of phenogenetic segregation of the various genotypes from Creeper matings, since this information is fundamental to a study of the action of the *Cp*-factor; 2) to give practical indications for early identification of these genotypes, as a necessary basis for any experimental work on early stages.

LANDAUER (1932) has shown that at 72 hours' incubation approximately 25 percent of the embryos from a Creeper × Creeper mating ($Cp + \times Cp +$) are markedly retarded in body size and differentiation. He has further shown that at 48 and even at 36 hours' incubation approximately one-fourth of the embryos fall into a developmentally retarded group—as regards both somite number and dimensions—a group almost discontinuous from the main population (table 5). The obvious inference is that it is this retarded group that represents the homozygous lethal segregates, and this has until now been the basis for early identification of these segregates.

In order to establish this criterion on a firm experimental basis, and to find if it holds for stages earlier than 36 hours' incubation, we undertook independently a series of experiments in which somites were counted at definite periods of incubation, and the subsequent development of the individual embryos observed. All embryos came from the same stock, which has been maintained at Storrs; $Cp + \times Cp +$ matings were used, as well as one mating of $Cp +$ hens with a $++$ cock. Experiments were carried on at Storrs and at St. Louis. At Storrs a large forced-draft incubator was used, maintained at 36 degrees centigrade with very slight fluctuations. At St. Louis, a Buffalo incubator, without forced draft, was used at 38–39 degrees, with some slight fluctuations above and below this range.

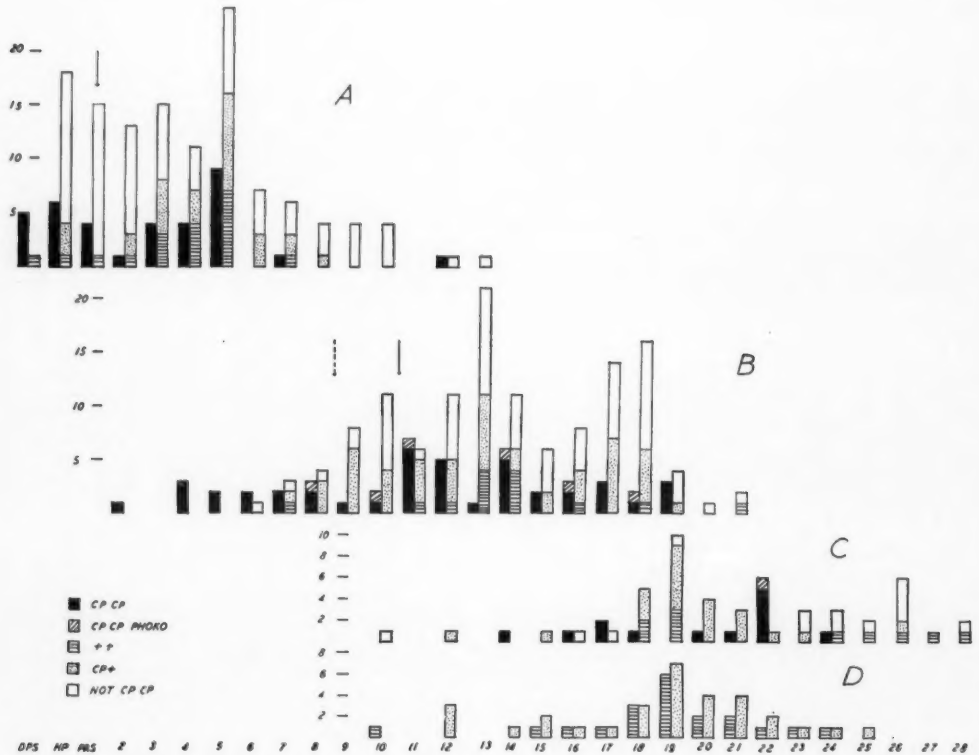

FIGURE 1.—Block diagrams showing distribution of somite stages among the segregates of $CP + \times CP +$ matings (A, B, C) and $CP + \times + +$ (D); A at 24–25 hours' incubation, B at 32–44 hours, C at 48–50 hours and D at 48 hours. DPS = Definitive primitive streak stage; HP = head-process; Pr S. = pre-somite; other stages defined by somite number.

For the somite counts, a window was made in the egg and the blastoderm was stained lightly with neutral red transferred from an impregnated agar plate. The somites were counted under a binocular microscope, and the egg closed and allowed to incubate further. Instruments and materials were of course sterilized. Final observations were made either at 3–4 days, which permits distinction between pathological and viable embryos, or at 8–10 days, which permits recognition of phokomelic, heterozygous and normal types respectively. The operative procedure clearly does not interfere with subsequent expression of these various characters. Only embryos showing the typical symptoms—early pathology or later phokomelia—were classified as *Cp Cp*; only typical chondrodystrophics as *Cp* +; doubtful cases have been eliminated from our tables.

RESULTS

Figure 1 is a block diagram summarizing the somite development of the various segregates at three stages of incubation. The first three distributions are for embryos from *Cp* + × *Cp* + matings. The first one (A) consists of embryos of 24–25 hours' incubation. The second one (B) groups cases examined at 32–44 hours' incubation. It was found that the range of somite variability in 36 hour embryos operated at various times in the spring and early summer was so great that additional cases both earlier and later than this standard stage could be added without increasing the spread. The third distribution (C) is of cases of 48–50 hours' incubation. Developmental stages are listed on the abscissae, number of cases on the ordinates, and at each stage two columns are shown, one of *Cp Cp* types (that is, early pathological and phokomelic forms) and a second including all viable segregates—*Cp* +, normal, and viable embryos opened too early to be classified. This latter category may include a small number of phokomelic homozygotes which look normal at 3–4 days.

Discrimination between Cp Cp and all other groups

a. Somite counts. In the 24–25 hour cases, the means have been calculated by simply assigning a number to each stage, beginning with the definitive primitive streak as stage 1, and later converting the mean back to the corresponding somite stage for the sake of uniformity. This procedure may be open to criticism as unduly distorting time-relations, but it must be recalled that the somite stages themselves are far from being evenly spaced on the developmental time-scale: to this unevenness we attribute, for example, the excessively large number of 5 somite stages. The means given here must be considered quite strictly as means of stages, not of time-classes. When calculated this way, the mean for *Cp Cp* embryos at 24–25 hours is 2.4 somites ±2.8 and that for all non-*Cp Cp* em-

bryos is 3.7 somites ±2.9. Hence the mean for homozygotes is slightly but far from significantly lower than that of normal embryos. The variance is much the same. There is then no natural break in distribution between the two groups.

The 32–44 hour cases show a similar result. Again the mean for *Cp Cp* embryos is lower (11.6±4.5 somites) than that of non-*Cp Cp* ones (13.8 ±3.4 somites) but the difference is clearly not significant. In this case the variance of the *Cp Cp* embryos is greater than that of the normal ones.

In both of the foregoing distributions it can be seen that the lower somite brackets have more than the expected 1:3 ratio of *Cp Cp* embryos. In the 48–50 hour group even this is not true. The *Cp Cp* mean is only slightly lower than that of the non-*Cp Cp* embryos (19.9±2.9 as against 21.0±4.0) and the variance is less, so that several cases of very low somite number are found to be non-*Cp Cp* embryos.

b. Other morphological symptoms. At this latter stage of incubation (48 hours and more) the morphological symptoms of the lethal effect begin to be observable; in a few of our cases we could predict from the first examination that an embryo would be of the *Cp Cp* type.

LANDAUER (1932, tables 7, 8) has described and analyzed by measurements the head-retardation in 72 hour *Cp Cp* embryos, and has shown that the posterior part of the body is less inhibited than is the head (his tables 1, 2). He has also described the abnormalities of the heart and circulatory system as well as of the limb regions. We have made some additional observations on the development of the lethal syndrome that may be of interest here.

Normal embryos undergo drastic morphogenetic changes in the 17–20 somite period (about 48 hours' incubation). The cephalic flexure increases from a slight bend to an angle of 90 and more degrees, and the forebrain elongates concurrently. Meanwhile the whole head rotates to lie on its left side, and the amnion closes over it. The vitelline arteries commence to consolidate, and rapidly become large conspicuous trunks. The heart becomes a loop. These changes show a certain independence of one another and of somite formation. For example, an embryo (from a normal mating) may have a completely rotated head without the amnion's covering it completely. Vitelline artery function may begin at any stage of this phase of head-morphogenesis and between the 18–20 somite stages. The establishment of the vitelline arteries is of course a conspicuous step in the transformation of the area vasculosa to a functional circulatory network, arising from the union of discrete blood islands.

The deficiencies of the *Cp Cp* embryos are primarily these: the head region begins showing retardation at the 17–20 somite stage; its flexure and rotation are delayed, although they eventually proceed, the flexure to at

least 90 degrees, the rotation to completion. The head does not grow in volume at anything comparable to the normal rate; by 72 hours' incubation microcephaly is striking. (See LANDAUER 1932, Plate XI figures 2–4; Plate XII figures 1, 8). Sections through the head at this stage show extreme thinning of the brain wall—sometimes to only two cell layers—as well as marked cellular degeneration in the nervous system. The head mesenchyme is also very deficient in quantity in the examples we have examined histologically.

Accompanying the diminished head growth is usually a striking asymmetry expressed in the eyes and the otocysts. At the time of rotation, the left side, that is, the side on which the embryo comes to lie, begins to be differentially retarded. The retardation may even precede rotation: in one case, a 20 somite embryo which had not yet undergone rotation and which showed other symptoms of the lethal effect, the left optic vesicle was seen to be slightly delayed as compared with the right, in making contact with the lateral head ectoderm. Following this stage, in *Cp Cp* embryos, the right eye continues development at about the same pace as the rest of the head, forming a small eye-cup and lens. The left eye, by 72 hours, usually has barely started to form a cup, and the lens remains a thickening continuous with the body wall, occupying a groove in the optic vesicle. In fixed preparations such lenses are apt to be everted and to project outside the body; this may be only an artifact due to the abnormal fluid pressures in the embryo. In the living state these left eyes appear as very small shrunken knots of tissue. The left otocyst may be similarly delayed in closure and retarded in growth.

The heart, as LANDAUER has pointed out, shows much variability, being retarded in form in a degree corresponding to the head retardation, but often being quite enlarged in size as compared to the normal heart of a similar morphological stage. This variability extends to the heart wall as well. Some cases we have examined histologically show a fairly good amount of myocardial tissue; others show absolutely no thickening even in the ventricle.

The thinning of tissues that is marked in the head-region at 60 or 72 hours is apparent also in the trunk, although so much more variable that posterior cross-sections in some examples look quite normal. The posterior neural tube is not necessarily thinned or degenerating. The somites are usually very deficient in cell mass; the process of segmentation itself is very little retarded by comparison, as LANDAUER shows and as the present results also indicate. The Wolffian ridge remains a thin layer, filled with mesenchyme, instead of being thickly packed with cells. Small limb buds may appear, delayed with reference to the rest of the body form.

While these events are occurring in the embryo, similar failures appear

in the vascular area. Although some attempt is made at forming vitelline arteries, and a feeble circulation may always be observed in the main channels, the peripheral blood islands never become incorporated in the network and no effective vitelline circulation is ever formed. The vascular area remains full of discrete blood clots, and the whole appearance becomes more and more anaemic as the blastoderm expands on the third and fourth days. In sections it is seen that endothelium is differentiated from the blood islands throughout the vascular area, but that these either do not interconnect, or connect only by very fine openings, so that the erythroblasts are not free to move, but remain in densely packed masses. Within the embryo too the circulation is defective; extravasation and clotting occur in the main channels, which are greatly distended; in sections various non-vascular cavities such as the amniotic space and the coelom are found full of erythrocytes.

During the fourth and fifth days of incubation, thinning and degeneration of tissues continues and fluid spaces enlarge progressively; the embryo becomes vesicular and shapeless. Hearts were found still beating as late as the fifth day. Complete necrosis probably ensues before the seventh day.

All these changes are foreshadowed in embryos of 48 or more hours which have reached the 20–21 somite stage without establishment of strong vitelline arteries. A few of our cases in the 48–50 hour group, as we have said, were such embryos; they subsequently developed the typical pathology. However, all *Cp Cp* embryos are not distinguishable at this stage, as can be judged from two cases of 22-somite embryos in which vitelline arteries were observed to be functioning. These two were subsequently found to be *Cp Cp* individuals. Hence the stage when all *Cp Cp* embryos are distinctly diagnosable must be put at the 24–25 somite period (48–54 hours' incubation). Lack of strong functional vitelline arteries is unmistakable at this stage of somite development, as is head retardation.

Discrimination between heterozygous and normal embryos

Figure 1 also presents somite stages for Creeper and normal embryos. Here the distribution is clearly parallel. In the 24–25 hour lot, the *Cp* + mean is 4.1 somites ± 2.0; that for normals is 3.6 somites ± 1.9. In the 32–44 hour group, the *Cp* + mean is 13.1 ± 3.4; the normal mean 13.8 ± 3.1. In the 48–50 hour group the *Cp* + mean is 19.5 ± 2.7 as against 22.3 ± 3.8 for the ++ embryos. The fourth distribution (D) on the table is the somite record for *Cp* + and ++ progeny in a mating of Creeper hens by a normal cock, from which the two sorts of offspring are expected in equal quantities. This series, incidentally, was made at approximately the same season (May-June) as most of the preceding 48–50 hour group, and it is

obvious that the spread of stages is approximately the same. In this lot the mean of the *Cp* + embryos is 19.9 ± 2.9 somites, that of ++, 21.0 ± 4.0 somites. There is possibly a slight tendency for the normal embryos to be more advanced than the heterozygous, but it is obvious that none of these differences in any way approaches significance.

To illustrate the reliability of the general table as indicating variability actually realized in small lots of eggs, we include summaries of two individual experiments. One (Storrs: May) consisted of 19 usable cases, examined at 24 hours. Of the 7 later found to be *Cp Cp*, one was in the definitive primitive streak stage, one pre-somite, one 2-, one 4- and three 5-somites respectively. One 5 somite embryo was found to be normal at 72 hours. *Cp* + embryos were found at 2, 3, 5, 6 somites, ++ ones at 3, 4 and 5 somites. The other experiment (St. Louis: February) was at 40 hours' incubation. *Cp Cp* embryos had 8 and 11 somites; one 9 somite embryo was found to be not *Cp Cp*; four 9 somite cases and one of 10 somites turned out to be *Cp* +. Thus in individual settings, as well as in the total population, *Cp Cp* embryos may be distributed throughout the whole range.

DISCUSSION

We conclude from the evidence presented above that in the stages and under the conditions employed, *heterozygous and normal* segregates from Creeper matings are not distinguishable on the basis of somite formation nor by other morphological criteria. Distinct macroscopic differences appear not earlier than on the 7th day (LANDAUER 1931).

Concerning the early segregation of *Cp Cp*-embryos and their viable sibs, LANDAUER (1932) states: 1) that homozygous embryos are smaller than normal ones, at least from the 36th hour of incubation on; 2) that "with regard to the number of somites the homozygous Creeper embryos lag most conspicuously behind the normal ones during the period when in normal development a rapid multiplication in somite number takes place (close of the 2nd day)" (p. 391). No striking difference in somite number was found at 72 hours of incubation.

Our data refer to somite numbers only. They show a slight shift toward the lower somite groups at 24–36 hours; this difference disappears at 48 hours. Thus we extend the continuous grouping of somite number shown by LANDAUER (1932, table 5) for 72 hour embryos back to the 48 hour stage, and we find that the artificial grouping in his 48 hour series is not in agreement with the constitution of the embryos. At 36 hours this grouping is not as strict a break as he suggests, but a statistical trend; the same is true at the 24 hour stage.

We have not collected data on size differences within our material. Some

of LANDAUER's data on size differences may require revision in view of the present findings. The 36 and 48 hour embryos classified as "homozygous" (table 4) are identified as such by the criterion of somite number only and may therefore include a higher percentage of "normal" embryos than is assumed by the author (p. 368).

The size differences found by him at these stages are probably only the expression of differences in developmental stage, superimposed of course on great individual variability. If, for example, we examine head length (the dimension showing greatest reduction in the retarded individuals: p. 374) in those embryos of the 36 hour group which had 12 somites and were classified as normal (table 4, column 2, last 6 cases), we find a variation of from 1.60 to 1.83 mm, with a mean of 1.71 ± 0.09. In the 48 hour embryos classified as *Cp Cp*—that is, those with 12–15 somites—the head length varies from 1.20 to 2.04 mm with a mean at 1.49 mm ± 0.07. The probability that these groups belong to the same normal distribution is between .4 and .3 (FISHER 1936, p. 128 and table 4), and hence the difference of the means cannot be taken as statistically significant. There is no demonstration in this material—even though a number of individual cases in the tables may suggest it—that 12–15 somite embryos of 48 hours' incubation are absolutely or differentially smaller than embryos that have taken only 36 hours to reach the same stage.

A reinvestigation of the matter of size difference of the various genotypes as contrasted with developmental stage would be especially desirable since LANDAUER's interpretation of the mode of action of the Creeper-factor as a primary growth retardation is partly based on these data on size differences in early *Cp Cp*-embryos. His data on 72 hour embryos (table 1 and 2) are not in doubt; however, they do not concern the essential point: whether or not growth differences *precede* the pathological abnormalities—both of which are strikingly apparent at 72 hours' incubation. This question cannot be considered as definitely settled until the search for micropathological changes has been extended to stages preceding the 17–20 somite stages using material of which the genetic constitution is checked.

For the practical problem of selecting *Cp Cp* embryos in early stages, counts of somite numbers are not reliable as far as the detecting of individual cases is concerned. Selecting in the lowest quarter of the population (the expected proportion of *Cp Cp*) in no instance gives even a 50–50 chance of obtaining a homozygote. From the table it can be seen that in the lowest one-fourth of the 24–25 hour group (marked off by the solid arrow) we have only about a 1:2 chance of selecting a *Cp Cp*. In the 32–44 hour group, the lowest one-fourth gives a similar ratio (16:27); if we select in the lowest one-eighth (to the left of the dotted arrow) the ratio rises to 13:8; embryos below 6 somites are all *Cp Cp*. This suggests that in a group

of approximately 36 hour embryos, averaging 13–14 somites, embryos that are greatly retarded (less than 6 somites) will probably be *Cp Cp*. In the 48–50 hour group, where one begins to find other morphological criteria of retardation, somite number ceases to be a differential in any sense.

As an example of the effect of selection, we may cite the ratios obtained by us individually. The expectation in the population is of course 1:3 for *Cp Cp*: non-*Cp Cp*; and 2:1 for *Cp* +:+ + among those surviving till 8–9 days. The Storrs material (D. R.) was taken without any selection whatever. The 32–50 hour St. Louis material (V. H.) was partly used for other experiments and a small fraction of the highest somite groups discarded. The ratios in the various groups follow:

	STORRS				ST. LOUIS			
	Cp Cp: non-*Cp Cp*		*Cp* +:++		*Cp Cp*:non-*Cp Cp*		*Cp* +:++	
24–25 hours	27	76	28	19	8*	48*	—	—
32–44 hours	23	65	19	10	25	61	30	4
48–50 hours	9	32	11	8	5	12	10	2
Cp+×++ 48 hours	—	—	30	21	—	—	—	—

* We are indebted to MR. J. CAIRNS for placing these data at our disposal.

Thus selection of low somite embryos in practice selects against non-*Cp Cp* and evidently for homozygous embryos.

Differences in head length are useless for practical purposes; they are too slight in view of the great variability in length and developmental stage in a given age group. The only reliable criteria are the pathological symptoms listed above, particularly the asymmetry in eye and otocyst and the failure to establish a proper vitelline circulation. They become apparent at the end of the second day but show a certain variation in the time of their first manifestation.

SUMMARY

Embryos from Creeper×Creeper (*Cp* +×*Cp* +) matings were examined for somite number at three stages of incubation: 24–25 hours, 32–44 hours, 48–50 hours. Some cases from a *Cp* +×+ + mating were also used at 48 hours. They were incubated farther and their phenotypic fate ascertained. The following conclusions are drawn:

1. There is no basis in somite number or morphology for distinction between the + + and *Cp* + segregates in these stages.

2. At the two earlier stages (24–25 hour, 32–44 hour) the *Cp Cp* segregates are on the average slightly less advanced in somite development than are their viable sibs, the mean somite number being 2.4 as compared with 3.7 at 24 hours; 11.6 as compared with 13.8 in the 32–44 hour group.

The distributions are entirely continuous, as is shown in figure 1. Selection in the lower quarter of these groups would give better than a 1:3 chance of obtaining a *Cp Cp* individual.

3. At 48–50 hours, somite number ceases to be a differential between the *Cp Cp* and the viable segregates. At this time, the first signs of pathological changes appear in the *Cp Cp* individuals. Some observations on the course of these pathological changes are offered in supplement of the descriptions of LANDAUER (1932).

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THE INTERRELATIONS OF TEMPERATURE, BODY SIZE, AND CHARACTER EXPRESSION IN DROSOPHILA MELANOGASTER[1]

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INTRODUCTION

DURING the past half-century, numerous investigations have shown that the exact expression of many wild-type and mutant characteristics of diverse organisms depends upon the temperature at which development takes place. Concurrently, studies on relative growth have frequently demonstrated a regular relation between the size of the whole individual and the degree of development of certain of its organs. Between these two fields of investigation, which have grown up quite independently, there is a bridge. It is supplied by the fact that in some organisms, such as the insects, adult size is related to the temperature prevailing during development. Consequently, the results of any study of the effects of temperature upon character expression in these forms are possibly complicated by the failure of size to remain constant at all temperatures.

The present paper constitutes a step in an analysis of the interrelations of temperature, body size, and character expression in an insect, *Drosophila melanogaster*. It will be shown that in the cases of polychaetoid and Dichaete, two "temperature-responsive" mutants of this species, a large part of the apparent "temperature effect" is under the conditions of these experiments bound up with changes in body size attendant upon the temperature change. Were the flies of the various temperature series of equal size, then the so-called "temperature effect" would be greatly reduced.

The principal conclusions of this paper were summarily reported at the Seventh International Genetics Congress.

THE POLYCHAETOID MUTANT

Flies homozygous for this mutant (*pyd*, $3\text{–}39 \pm 2$) are characterized by the presence of extra bristles at or near the various normal bristle loci. Supernumerary macrochaetae are particularly likely to occur in the dorsocentral and scutellar regions. A detailed description of the mutant will be given by NEEL (1940). The stock employed in this investigation had been inbred in small mass cultures (three-four pair matings) for two years prior to the beginning of the experiments.

[1] This and a following paper (Genetics, in press) have been submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Rochester.

The size of the fly and the expression of polychaetoid, at constant temperature

The relation between the size of *pyd* flies and the number of bristles present has been investigated in several different ways. Thus, in a first approach to the problem, groups of *pyd* flies were raised under such unlike nutritional conditions as to result in differences in average fly size from group to group, and the relation between mean size, as expressed by body weight, and the mean number of dorsocentral bristles present per side per fly (M dc) established. As a standard procedure, two or three pairs of flies were introduced into half-pint milk bottles containing approximately 50 cc of corn-meal agar and allowed to remain there seven to eight days. The bottles were kept in an incubator similar to the two-shelf type described by BRIDGES (1932), in which temperature stayed within 0.2° of 24.0°C. When the offspring began to emerge the flies were collected at three day intervals. From one to four collections were made from a set of bottles, and thus groups of flies developing at different nutritional levels of the cultures and hence of different mean sizes were secured. The flies of each collection period were given unlimited access to food during the intervals between collections. Immediately after collection, all the males were etherized and weighed together, and the average body weight established. Only males were used in the weight determinations, since their weight is less subject to fluctuation than that of the females. For all groups the time elapsing between removal from food, etherization, and weighing was made as short as possible (ca 10–15 minutes). This minimized errors due to dehydration of the flies. The M dc was then calculated. A total of ten groups of flies, representing a range in average fly weight of from 0.600 ± 0.011 to 0.953 ± 0.004 mg, and in M dc of from 2.322 ± 0.052 to 3.470 ± 0.034, was thus secured. The mean number of males weighed in a group was 98.7; an average of 139.6 half thoraces from these were used in the M dc determinations. The complete data for this experiment and for the similar experiments to be reported below have been filed with GENETICS.

A simple expression for the relation between average body weight and M dc was desired. It was found that while a plot of M dc against average body weight yielded an approximation to a straight line, a better approach to linearity was secured with a double log plot (figure 1). This indicates that the data can be treated as an expression of the power function

$$y = bx^k$$

recognized by biologists as the relative growth function. In this case, y corresponds to M dc, x to mean body weight, and b and k are constants.

The ranges of the variables x and y are towards the lower limits of valid applications of logs.

The line which has been fitted to the points of figure 1 is the regression line of log M dc on log mean body weight, as determined by the least squares method with unweighted ordinates. The equation of this line is

$$Y = 0.713X + 0.577$$

where Y corresponds to log M dc and X to log mean body weight. The aberrant point seen in the lower left hand corner of the figure has not been

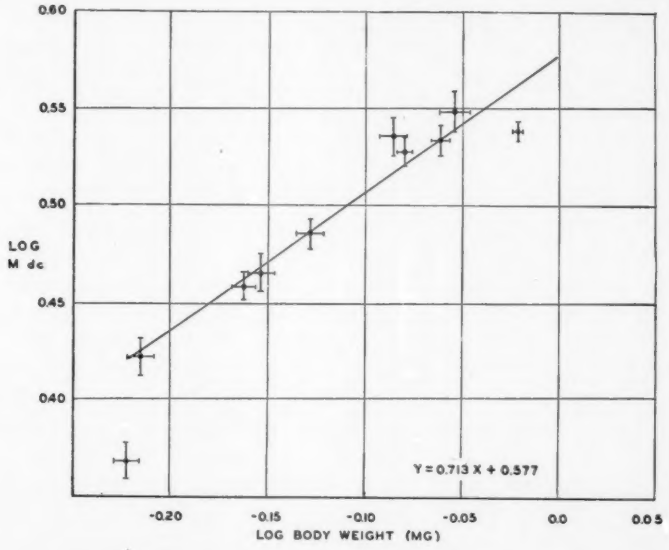

FIGURE 1.—The correlation, in groups of male *pyd* flies raised at 24.0°C at various nutritional levels, between log average body weight and log M dc. The vertical lines attached to each point represent the limits of $\pm\sigma_m$ for the M dc. The horizontal bars indicate the calculated magnitude of $\pm\sigma_m$ for the average weight. All logs are to the base 10. Details in text.

included in the fitting of the line, since for reasons unknown it is in such obvious disagreement with the others. The flies from which the extreme right hand point in the figure was derived were raised under somewhat different conditions than the rest, namely, from eggs laid over a two to six hour period and allowed to develop in finger bowls of 10.5 cm diameter containing approximately a one centimeter layer of agar, one hundred to a bowl.

Each of the points shown in figure 1 is based upon two means, M dc and mean body weight. The determination of these means involves both methodological and sampling errors. In consequence of the simplicity of the operations involved (counting bristles and weighing groups of ca 50–150 flies on a Becker Chainomatic Balance with a sensitivity of 0.2 mg), methodological errors are small. Sampling errors are of somewhat greater magnitude. The sampling error for bristle number is given by the standard error of the M dc (σ_m). The upper and lower limits of the vertical bar attached to each point of figure 1 are respectively M dc$+\sigma_m$ and M dc$-\sigma_m$.

The evaluation of the sampling error for weight, which is the standard error of the mean body weight of a group, is somewhat more difficult. In the course of the experiments reported in this paper, two different methods have been used in raising groups of flies of different average sizes. a) The one method, used in securing the flies incorporated into figure 1, involved the withdrawal from culture bottles of flies emerging during successive three day periods, and has already been described in some detail. b) The other method, used in the derivation of the points of figures 3, 4, 5, and 6, was to place varying numbers of eggs from two to eight hour egg-laying periods on a given quantity of food (amount contained in a one centimeter layer of corn-meal agar in a finger bowl of 10.5 cm diameter). It seems that the first method should lead to a somewhat greater variation in body weight within the group than the second. Accordingly, the means of estimating the weight error which follows is based upon flies raised by the first procedure; this should be adequate for flies raised by the second method.

One hundred male flies composing a portion of the first three days emergence from culture bottles subjected to standard conditions were weighed individually on a microbalance with a sensitivity of 0.001 mg. Repeated weighings of the same fly showed that the accuracy of the weight determination was within one percent. For these 100 flies, the mean body weight (M) was 0.881 mg; the standard deviation (σ) was 0.089 mg; the standard error of the mean (σ_m), 0.009 mg; and the coefficient of variability (CV), 10.1. From these determinations, an estimate of the error of the weight determination for each group can be made. It is assumed that from group to group, no matter what the average body weight, CV remains approximately constant and equal to the value found in these 100 flies. Hence in any other group, σ_m may be reasonably estimated as equal to .01 $M/\sqrt{N}$, since $\sigma_m = \sigma/\sqrt{N} = (CV \times M)/100\sqrt{N}$. The standard errors so estimated are indicated in figure 1 by horizontal bars.

Some of the points in figure 1 deviate significantly from the fitted line, where the criterion of significance is deviation by an amount equal to three

times the standard error of body weight or M dc. Similar deviations will be noted in later figures. Possible causes for these deviations are as follows: 1) The relation between the two sets of values studied may not be strictly linear. Thus, HUXLEY (1932) has pointed out that in cases of insect heterogony, at large body sizes points usually fall below the straight line fitted to a double log plot of the data. A deviation which may be of this nature is shown in the figure. 2) Experience has shown that the weight of a given group of flies may from time to time vary considerably, depending on feeding conditions. In this work, an attempt was made to give each group of flies the same free access to food, prior to weighing. It is possible that this attempt was not entirely successful, and that therein lies the cause of at least part of these deviations.

A second determination of the relation between size and dorsocentral number consisted in deriving the correlation in individual flies between femur length and total number of dorsocentrals present. Femur length has been used as an index to body size by a number of investigators (cf BREHME 1939), but the exact relation between these two variables has not been established. Since an investigation of this point seemed desirable, the right fore-femurs of the males composing each of the ten samples incorporated into figure 1 were mounted, and the mean femur length of each sample determined from camera lucida drawings of the mounted femurs. Over a range in mean femur length from 516.0 ± 4.55 to $582.0 \pm 3.16\mu$ and in mean body weight from 0.600 ± 0.011 to 0.953 ± 0.004 mg, these two variables appeared to be linearly related. It seems probable that with uniform age and feeding this group relation would hold good for the individual flies which compose the group.

Male flies were collected at intervals over a period of three weeks from cultures kept at 24.0°C, in order to get a considerable distribution in size. The right fore-femur and the dorsum of the thorax of each fly considered were mounted together on a slide. For this population ($N = 134$), the correlation between femur length and total number of dorsocentral bristles present on both sides of the fly was 0.619 ± 0.053. There was a still higher higher correlation between femur length and the total length of the dorsocentrals present (0.705 ± 0.044). To determine this latter measurement the bristles were drawn by camera lucida (see NEEL 1940). It seems probable that slight errors of measurement tend to obscure an even greater natural correlation between these variables. A similar strong correlation between the size of individual flies (as measured by over-all length) and dorsocentral number, in an extra-bristled stock, has been reported by MACDOWELL (1915).

The third method of evaluating the relation between size and bristle number consists in correlating the weight of the individual fly with the

number of dorsocentrals present on both sides of the fly. In the sample of 100 *pyd* males which were weighed individually to determine the standard error of mean body weight (p. 228), the correlation was 0.278 ± 0.092. An insignificantly higher value (0.308 ± 0.090) is obtained when the logs of the two variables are correlated. This correlation between size and bristle number, although significant, is much looser than that indicated by the other two approaches. There are two outstanding reasons for this. 1) The range in body size in these 100 flies was not as great as that utilized in the

TABLE 1

The relation between length of egg-larval life and number of dorsocentral bristles present in male pyd flies, when size is held constant.

a. Femur length between 569 and 581μ				
Interval after oviposition at which puparium formation occurred (hours at 21°C)	132.0–139.9	140.0–147.9	148.0–155.9	156.0–163.9
Number of flies	28	35	59	19
Mean total number of dorsocentrals $\pm \sigma_m$	6.71 ± 0.21	6.68 ± 0.15	6.68 ± 0.12	6.64*
b. Femur length between 582 and 594μ				
Interval after oviposition at which puparium formation occurred (hours at 21°C)	132.0–139.9	140.0–147.9	148.0–155.9	156.0–163.9
Number of flies	26	77	41	8
Mean total number of dorsocentrals $\pm \sigma_m$	7.11 ± 0.21	6.72 ± 0.11	7.00 ± 0.12	6.75*

* No σ_m calculated because of the small number of flies involved.

other two experiments. Since a correlation between two variables becomes more apparent as the range of either is extended, the smaller size distribution probably contributes to the relatively low correlation observed. 2) The flies weighed were those emerging in the first three days from a set of bottles subject to conditions described above. The weighings required two days. Although every effort was made to give the flies unlimited access to food, microscopic investigation at the time of weighing revealed considerable differences from fly to fly in the degree of distension of the abdomen. These are in part due to age differences. Because of this variation in abdominal contents, body weight must be regarded as a less accurate index of innate fly size than femur length.

In a group of flies, random weight fluctuations from fly to fly would be expected to cancel out, so that average body weight for the group, as compared with that for other groups, would probably be an accurate index of

mean size. This appears a reasonable explanation for the fact that for different groups of flies the correlation between average body weight and M dc is quite high (figure 1), although for individual flies the correlation between weight and total number of dorsocentrals is much lower.

It is apparent from each of these three procedures that at a constant temperature bristle number is linked with body size; an estimate of the closeness of the association depends upon the particular approach used.

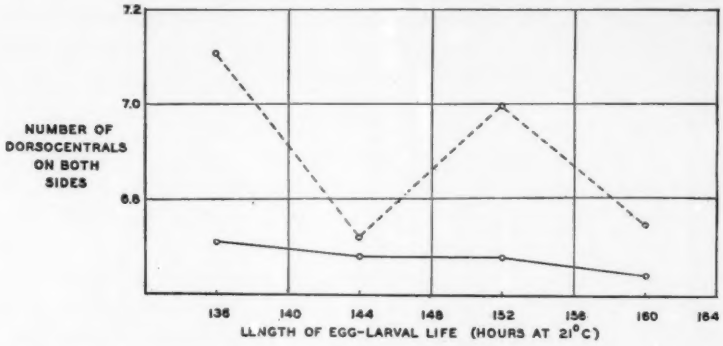

FIGURE 2.—The lack of correlation of length of egg-larval life with the total number of dorsocentral bristles present in *pyd* flies. The circles connected by the broken lines represent groups of flies with femurs 582–594μ long, while those connected by solid lines are based on flies with femurs 569–581μ in length. Details in text.

Independence of M dc and length of larval life, at constant temperature

Groups of flies of different average sizes have been obtained by varying cultural conditions. Thus, some of the groups used in deriving the regression equations developed at quite low nutritional levels. A concomitant of a restriction on food intake such as practiced here is an increase in the average duration of larval life. It seemed necessary to test for a possible correlation of this prolongation of the larval stadium with bristle number, for the existence of such a relation would complicate any interpretation of the size-bristle number relation. Accordingly, an experiment was designed which would permit a comparison of the total number of dorsocentrals in equal-sized flies whose larval lives had been of unequal lengths.

Eggs laid by *pyd* females over a two-hour period were transferred to finger bowls containing yeasted corn-meal agar; each bowl received sufficient eggs that the resulting larvae developed under sub-optimum food conditions. This insured a considerable distribution in the time of puparium formation. Development took place at 21°C. The great majority

of puparia were formed from 132 to 164 hours after oviposition. New puparia were isolated every four hours during this period. When the flies emerged, the males of each collection period were classified as to the total number of dorsocentrals present and the right fore-femur length. A total of 527 flies was treated in this manner. Some males with femurs between 569 and 594μ in length were found in each collection. The mean total number of dorsocentrals of flies having femurs 569 to 581μ long was calculated for each four-hour group. A similar calculation was made for flies whose femurs ranged in length between 582 and 594μ. Since this subdivision of the data meant that the number of cases in any four-hour group was small, adjacent groups have been combined, so that there are available four eight-hour periods.

The data are given in table 1. In figure 2 for each of the two femur length ranges (= fly sizes) mean number of dorsocentrals has been plotted against length of larval life. For each eight-hour group, the length of larval life has been treated as the mid-point of that period. The broken line connects circles representing groups of flies with femurs 582–594μ long; the circles connected by the solid lines are based on flies with femurs 569–581μ in length. No significant trend in the data is evident. This suggests that the inequalities in length of larval life observed at the various nutritional levels are of no significance in the determination of bristle number.

The relation between temperature and M dc

To test the effect of temperature upon fly size and M dc, well fed *pyd* females were allowed to oviposit at room temperature on food exposed in small rectangular metal containers. The eggs obtained in a two to eight-hour egg-laying period were divided into lots of 100 and placed on well

TABLE 2

The effect of temperature upon bristle number and body weight in pyd Drosophila melanogaster males. M dc stands for the mean number of dorsocentrals present per side per fly.

TEMPERATURE (°C)	M dc $\pm\sigma_m$	NO. HALF THORACES	AVERAGE BODY WEIGHT (MG $\pm\sigma_m$)	NO. FLIES WEIGHED
14.0°	4.533±0.033	542	1.061±0.006	276
19.0°	4.213±0.023	754	1.062±0.005	441
24.0°	3.470±0.034	536	0.953±0.004	500
29.0°	3.058±0.022	740	0.784±0.003	396

yeasted corn-meal agar contained in finger bowls of 10.5 cm diameter. The bowls were then put at the various experimental temperatures. Such a procedure should insure each larva a plentiful supply of food. Subsequent to puparium formation, the puparia were placed in vials furnished with enough food that the flies could feed freely after emergence.

The temperatures at which the flies were raised and the M dc and average fly weight at each temperature are given in table 2. A 15°C increase in temperature is associated with a pronounced decrease in the M dc, from 4.533±0.033 at 14°C to 3.058±0.022 at 29°C. Simultaneously, however, mean weight is decreasing. In view of the above-demonstrated correlation between fly size and M dc, a question immediately arises. To what extent is this effect of temperature upon bristle number concerned with some bristle-forming process within the body not related to fly size, and to what extent is the effect mediated either through size or some processes common to size and M dc?

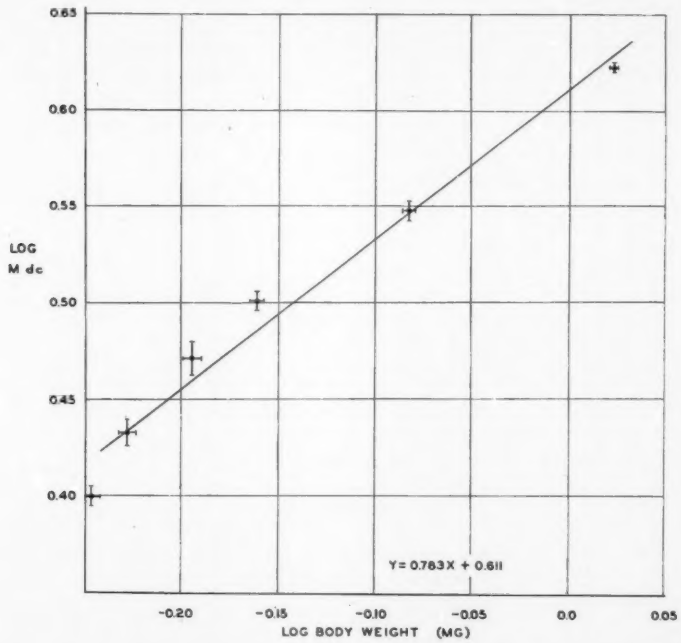

FIGURE 3.—The relation, in groups of *pyd* flies raised at 19.0°C at various nutritional levels, between log average body weight and log M dc.

An evaluation of the manner of action of temperature

Given that there is a change in the M dc from one temperature to another, then let a direct effect of temperature upon bristle number be defined as one which is independent of the size differences at the various temperatures, while an indirect effect is one which under these experimental conditions is bound up with a size change. The question raised in

the last paragraph may be restated thus: what are the relative roles of these two effects in the total temperature effect?

To answer this question, equations for the regression of M dc on weight at each of the three lower temperatures employed are necessary. This equation has already been obtained at 24.0°C (p. 227). The relation at the other two temperatures was determined by placing egg samples from two to eight-hour laying periods under various food conditions (different numbers of eggs per finger bowl containing food). Groups of flies showing wide differences in average body weight were secured.

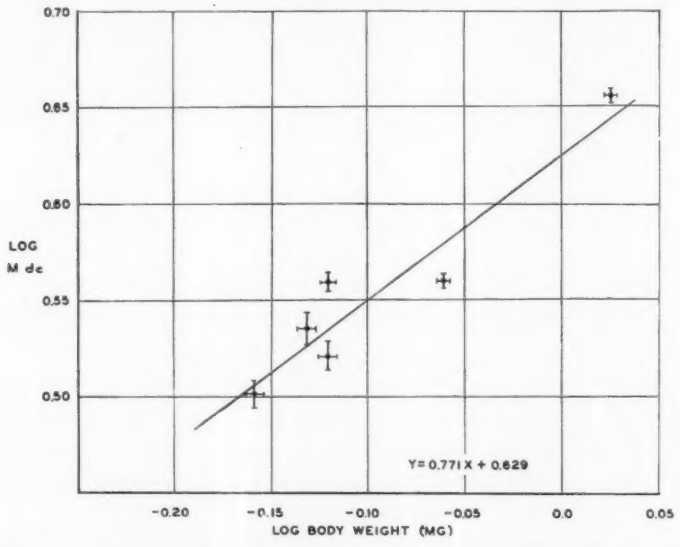

FIGURE 4.—The correlation between log average body weight and log M dc, in groups of *pyd* flies raised at 14.0°C at different nutritional levels.

Six lots of flies were raised at 19.0°C. The highest mean body weight secured for any group was 1.062 ± 0.005 mg; this was accompanied by an M dc of 4.213 ± 0.023. These were the flies which have already been discussed, raised under optimum conditions and listed in table 2. The lowest mean body weight was 0.568 ± 0.005 mg; the corresponding M dc was 2.508 ± 0.035. In agreement with the 24.0°C data, a working approximation to a linear relation is observed when log M dc is plotted against log average body weight (figure 3). The equation for the regression of log M dc on log weight is

$$Y = 0.783X + 0.611.$$

Six groups of flies, varying in weight from 1.061 ± 0.006 to 0.694 ± 0.007 mg and in M dc from 4.533 ± 0.033 to 3.175 ± 0.049, were raised at 14.0°C. The distribution of the data is such that it would be difficult to determine the exact nature of the regression. However, on the assumption that it is not different from that believed to obtain at 24.0° and 19.0°C, a straight line of best fit to a double log plot of the results has been obtained (figure 4.) Its equation is

$$Y = 0.771X + 0.629.$$

Possible causes for the significant departure of some points from the fitted line have already been discussed.

These regression equations make possible a determination of the relative importance of direct and indirect temperature effects. Two methods of evaluation have been employed. The first is summarized in table 3. In

TABLE 3

An evaluation of the relative importance of direct and indirect temperature effects in the expression of the pyd mutant of Drosophila melanogaster.

A	B	C	D	E	F	G	H
TEMPERATURE RANGE	OBSERVED M dc AT LOWER TEMPERATURE	OBSERVED M dc AT HIGHER TEMPERATURE	DIFFERENCE B−C	CALCULATED M dc AT LOWER TEMPERATURE*	DIFFERENCE B−E (=INDIRECT TEMPERATURE EFFECT)	DIFFERENCE D−F (=DIRECT TEMPERATURE EFFECT)	RATIO OF F/G
29°–24°	3.470	3.058	0.412	3.177	0.293	0.119	2.46
29°–19°	4.213	3.058	1.155	3.373	0.840	0.315	2.66
29°–14°	4.533	3.058	1.475	3.532	1.001	0.474	2.11
24°–19°	4.213	3.470	0.743	3.936	0.277	0.466	0.59
24°–14°	4.533	3.470	1.063	4.102	0.431	0.632	0.68
19°–14°	4.533	4.213	0.320	4.457	0.076	0.244	0.31

* If size equals maximum size at higher temperature.

column A of the table the six temperature combinations possible from these data have been listed. Column B shows the M dc at the lower of the two temperatures composing a given combination, while column C gives the M dc at the upper of the two. Column D, showing the difference B−C, gives the total temperature effect on the M dc over the given temperature range. From the regression equations which have been developed, it has been calculated what the M dc would be at each of the lower temperatures, if average fly size were equal to that obtaining at the higher temperatures. The results of these calculations are entered in column E. The difference between the entries in columns B and E, given in column F, is the change in M dc correlated under these conditions with the size change

(indirect effect). The difference between the entries in columns D and F (or E−C), given in column G, is the change in M dc independent of a size factor (direct effect). Finally, the ratio of the entry in column F to that in G (column H) is the ratio of indirect to direct temperature effects over the given range. It appears that under these experimental conditions the major portion of the effect of temperature upon the M dc is indirect. The role of the indirect effect is not the same at all temperatures, but is greatest at high temperatures (where the M dc is changing most rapidly) and least at low.

In the above procedure, in columns B and C have been listed the actually observed M dc at the observed maximum size. This M dc does not always correspond to that expected on the basis of the regression equation. This is of course due to the circumstance that not all points fall right on the regression line. In this evaluation it is possible to use, not the actual observed M dc at maximum size, but the M dc which as calculated from the appropriate regression equation should accompany this size. This modification of the procedure results in a slightly higher average value for the ratio of indirect to direct effects. If the deviation below the line of the terminal points in the 24.0° and 19.0°C results is a systematic rather than a random occurrence, then this modification is less valid than the original procedure.

A second approach to this evaluation problem has been developed by DR. D. R. CHARLES of this laboratory, who has kindly given me permission to incorporate the method and its results into this paper.

EIGENBRODT (1930) has shown that as temperature decreases, weight increases, down to ca 17°C; "below 17°C there is very little increase in weight" (p. 395). Similarly, RIEDEL (1934) has found that the tibia length of wild-type flies increases with temperature decrease until the 15–16°C point is reached. With further reduction in temperature, tibia length now decreases. The data contained in the present paper (see also below) tend to confirm these observations, that in the neighborhood of 15°C fly size is at a maximum. On the assumption that weight is an exponential function of temperature deviation from 15°C, the following equation has been found to describe satisfactorily the observed relation of temperature to body weight, under optimum larval feeding conditions:

$$\log W = 0.0373 - 0.0007\,|T - 15|^{2.041}. \qquad (1)$$

In this equation, W corresponds to body weight and T to temperature.

The M dc is a function of both size and temperature. The equation derived from the preceding data which relates these three variables is:

$$\log B = 0.640 + 0.800 \log W - 0.0069[T - 15]^{0.990}. \qquad (2)$$

Here W and T have the same significance as in (1), and B represents M dc.

Equations (1) and (2) may be written as

$$W = 1.0898\ e^{-0.0015|T-15|^{2.641}} \tag{3}$$

and

$$B = 4.365\ W^{0.800}e^{-0.0159[T-15]^{0.990}}. \tag{4}$$

For convenience in the following discussion, these equations will be represented by

$$W = k_1\ e^{k_2|T-15|^{k_3}} \tag{5}$$

and

$$B = k_4\ W^{k_5}e^{k_6[T-15]^{k_7}}. \tag{6}$$

From equation (5) it follows that at any given temperature, the rate of change in body weight is given by

$$\frac{dW}{dT} = k_1k_2k_3\,|T - 15\,|^{k_3-1}e^{k_2|T-15|^{k_3}} \tag{7}$$

and the amount of change, ΔW, over a small temperature interval, ΔT, is

$$\Delta W \doteq k_1k_2k_3\,|T - 15\,|^{k_3-1}e^{k_2|T-15|^{k_3}}\Delta T \tag{8}$$

which by substitution from (5) becomes

$$\Delta W \doteq k_2k_3\,|T - 15\,|^{k_3-1}W\Delta T. \tag{9}$$

Equation (6) is of the form $B = f(W, T)$. As a result of partial differentiation of this equation with respect to W, it is seen that as temperature changes, at any instant the rate of change in the M dc linked with the size change may be expressed as

$$\frac{\partial B}{\partial W} = k_4k_5W^{k_5-1}e^{k_6[T-15]^{k_7}} \tag{10}$$

and the amount of change in the M dc linked with the size change (ΔB_W) as

$$\Delta B_W \doteq k_4k_5W^{k_5-1}e^{k_6[T-15]^{k_7}}\Delta W. \tag{11}$$

This by substitution from equation (9) and then (6) and simplification becomes

$$\Delta B_W \doteq k_2k_3k_5\,|T - 15\,|^{k_3-1}B\Delta T. \tag{12}$$

Similarly it is seen that the rate of change in the M dc due directly to the effect of temperature change may be expressed by

$$\frac{\partial B}{\partial T} = k_4 k_6 k_7 W^{k_5} [T - 15]^{k_7 - 1} e^{k_6 [T-15]^{k_7}} \tag{13}$$

while the amount of change in the M dc due directly to the temperature change (ΔB_T) is

$$\Delta B_T \doteq k_4 k_6 k_7 W^{k_5} [T - 15]^{k_7 - 1} e^{k_6 [T-15]^{k_7}} \Delta T. \tag{14}$$

This by substitution from equation (6) becomes

$$\Delta B_T \doteq k_6 k_7 [T - 15]^{k_7 - 1} B \Delta T. \tag{15}$$

Over a vanishingly small temperature interval, the ratio of the amount of change due to an indirect effect of temperature to the amount due to a direct effect is

$$\frac{\Delta B_W}{\Delta B_T} = \frac{k_2 k_3 k_5 |T - 15|^{k_2 - 1} B \Delta T}{k_6 k_7 [T - 15]^{k_7 - 1} B \Delta T} = \frac{k_2 k_3 k_5}{k_6 k_7} |T - 15|^{k_2 - k_7}. \tag{16}$$

After substitution of the numerical values of the above constants, which are given in equations (3) and (4), equation (16) reduces to

$$\frac{\Delta B_W}{\Delta B_T} = \frac{|T - 15|^{1.05}}{6.25}. \tag{17}$$

In order to evaluate the ratio of indirect to direct temperature effects over an infinitesimal temperature range at any given temperature, it is necessary only to substitute that given temperature for T of the equation. This substitution has been carried out at 1°C temperature intervals over the whole range employed (14.0–29.0°C). The results are given below.

T	14°	15°	16°	17°	18°	19°	20°	21°	22°	23°
$\frac{\Delta B_W}{\Delta B_T}$	0.16	0.0	0.16	0.33	0.51	0.69	0.87	1.05	1.23	1.42

24°	25°	26°	27°	28°	29°
1.61	1.80	1.98	2.17	2.36	2.56

Here again, in agreement with the results given in table 3, it is found that under these conditions the role of the indirect effect is greatest at high temperatures. The chief difference between the results of the two evaluations is that the second method consistently assigns to the indirect effect a smaller value than does the first.

THE DICHAETE MUTANT

These findings concerning the role played by a size factor in the *pyd* temperature effect made it desirable to determine whether similar relationships exist for other temperature-responsive mutants. After a pre-

liminary survey of the literature the *Drosophila melanogaster* mutant Dichaete (*D*, 3–40.4–41.0±) was chosen for an analysis of the type conducted on *pyd*. BRIDGES has shown that this mutant is associated with a short inversion of 3L (MORGAN, BRIDGES, and SCHULTZ 1937). The character is dominant, lethal when homozygous, and in addition to other morphological effects is characterized by a reduction in the number of chaetae present. The dorsocentral bristles are particularly likely to be affected. Either one or both of the two normally present on a side may be missing. When only one is absent, it usually corresponds to the anterior dorsocentral. Occasionally, when one bristle is missing, the remaining does not correspond strictly to either the anterior or posterior dorsocentral, but is located at some point between the usual positions of these two. The character was studied in a balanced Lyra/Dichaete line.

PLUNKETT (1926) found that with an increase in temperature there is a marked decrease in the mean number of posterior dorsocentrals present per side per fly (M p dc) in Dichaete flies. At 24.5°C, where the duration of the egg-larval-pupal period is 9.5 days, temperature was reported to have an effect upon bristle number from the second to the eighth days. Puparium formation was found to mark the approximate mid-point of the temperature-effective period.

TABLE 4

The relation of bristle number and body weight to temperature in Dichaete Drosophila melanogaster males. M dc represents the mean number of dorsocentral bristles present per side per fly.

TEMPERATURE (°C)	M dc $\pm\sigma_m$	NO. HALF THORACES	AVERAGE BODY WEIGHT (MG)	NO. FLIES WEIGHED
14.0°	1.530±0.025	268	0.914	137
19.0°	1.557±0.014	788	0.922	417
24.0°	1.356±0.018	480	0.864	243
29.0°	1.213±0.015	632	0.725	325

In the present work the criterion of size and temperature effects has been the mean frequency of occurrence of both the anterior and posterior dorsocentrals (M dc), rather than the mean frequency of occurrence of the posterior dorsocentral alone (M p dc) studied by PLUNKETT. This is due to the occasional difficulty, when only one dorsocentral is present, in definitely identifying it as anterior or posterior.

The relation between temperature and M dc

Lyra/Dichaete flies of a long-inbred strain were raised at four different temperatures. The procedure was as described for the similar *pyd* experiment (p. 232). Table 4 shows that the M dc increased from 1.213±0.015 to

1.557 ± 0.014 when the temperature decreased from 29.0° to 19.0°C. However, an additional 5°C decrease, from 19.0° to 14.0°C, was without significant effect upon bristle number. It is of interest to note that the 19.0°C flies are 27 percent heavier than the 29.0°C group, and also slightly heavier than the 14.0°C group. Already in these data there are indications of a parallelism between size and M dc. PLUNKETT (1926) found that in his stock a temperature decrease over the lower part of the range employed did have an effect upon M dc, since the M p dc at 20.0°C was .439 ± 0.013, at 17.0°C 0.581 ± 0.023, and at 14.0°C 0.690 ± 0.036.

The correlation between fly size and Dichaete expression, at constant temperature

Groups of *D* flies were raised at a constant temperature but at different nutritional levels and the relation between M dc and average weight determined. The *D* relation appears to be different from that observed for *pyd*. Whereas in the latter instance an approximation to a straight line was obtained when log M dc was plotted against log average body weight, in the former a straight line relation was observed with a semi-log plot. This is evident from figure 5, where for eight groups of flies of different average sizes raised at 24.0°C, M dc has been plotted against log mean body weight. The equation of the regression line fitted to these points is

$$Y = 3.606X + 1.637$$

where Y corresponds to M dc and X to log mean body weight. The meaning in this apparent difference in the relation of M dc to weight in *pyd* and *D* is not at present clear.

In figure 6 is shown the 19.0°C regression of M dc on body weight. As at 24.0°C, so here an approximation to a straight line is obtained when log weight is plotted against M dc. The equation of the line of best fit to these data is

$$Y = 3.337X + 1.705.$$

The standard error of mean body weight has not been indicated in these figures. The calculation of this constant by the method given earlier demands data on the CV of individual body weight, and such data were not available for Lyra/Dichaete flies.

The flies employed in this work grew very poorly at 14.0°C. Although a number of different egg samples were started under various cultural conditions, only two yielded adults in sufficient numbers to give a reliable M dc and mean body weight. This circumstance makes impossible the derivation of a regression equation at 14.0°C. One of the two groups which did come through at this temperature developed under very favorable

conditions; the M dc and weight for these flies has been given in table 4. By a fortunate coincidence, flies of the other group, grown under less favorable conditions, had an average body weight of 0.726 mg, which is almost identical with the body weight of the 29.0°C flies. The M dc for this group was 1.139 ± 0.021.

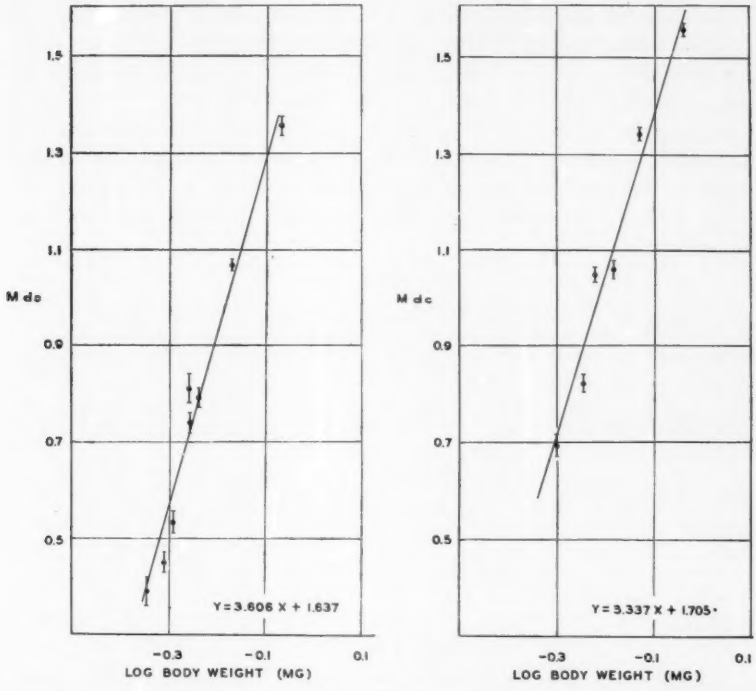

FIGURE 5 (left).—The relation, in *D* flies, between M dc and log average body weight. Each point represents a group of flies raised at a particular nutritional level at 24.0°C. The limits of plus or minus one times the standard error of the M dc are indicated by vertical bars, as before.

FIGURE 6 (right).—As above, but at 19.0°C.

The relation between M dc and length of larval life, at constant temperature

As in the *pyd* case, so for *D* it is necessary to determine whether the increase in length of larval life at lower nutritional levels is correlated with the M dc. PLUNKETT (1926) noted that in *D* flies malnutrition resulted in a decrease in the M p dc, that males have a smaller M p dc than females, and that the introduction of a Minute mutant into a *D* line resulted in a

lowered M p dc. Each of these three factors (malnutrition, maleness, Minutes) was observed to "decrease the size of the flies as well as the rate of development and the mean bristle numbers" (p. 229). Although it was recognized that the data then available were inadequate to analyze the interrelations among these three phenomena, it was thought more probable that the observed decrease in the M p dc was correlated with the increase in the length of developmental time than with the size decrease.

Lyra/Dichaete eggs laid over a two-hour period were placed at 19.0°C under somewhat overcrowded conditions. When puparium formation began, new puparia were isolated every four hours. Each fly after emergence was classified as to femur length and total number of dorsocentrals, and the bristle numbers of equal sized flies compared. The data are given in table 5. Within the limits of this experiment there is no evidence that length of larval life is correlated with the number of dorsocentrals present in *D* flies.

TABLE 5

The absence of correlation between length of egg-larval life and total number of dorsocentral bristles in Dichaete flies, when size is held constant.

INTERVAL AFTER OVIPOSITION AT WHICH PUPARIUM FORMATION OCCURRED (HOURS AT 19°C)	SEX	FEMUR LENGTH	NUMBER OF FLIES	TOTAL NUMBER OF DORSOCENTRALS $\pm\sigma_m$
194.0–210.9	♀♀	580–606μ	28	2.964±0.131
211.0–234.9	♀♀	580–606μ	23	3.087±0.168
194.0–210.9	♂♂	561–587μ	37	2.432±0.096
211.0–234.9	♂♂	561–587μ	26	2.269±0.164

An evaluation of the manner of action of temperature

The roles of indirect and direct temperature effects may now be briefly evaluated (figure 7). The upper, solid line of figure 7 connects circles showing the values obtained for the M dc at each of the four experimental temperatures when fly size was maximum at that temperature. Circles connected by the lower, broken line indicate M dc values to be expected at the same four temperatures if flies were the same (29.0°C) size at all temperatures. The four points connected by this lower, broken line are: 1) The M dc obtained for the 29.0°C flies, 2) the M dc which the regression equation indicates would be found at 24.0°C if average weight were equal to that of the 29.0°C flies, 3) the M dc which from the regression equation would be expected at 19.0°C if average weight were the same as observed at 29.0°C, and 4) the M dc actually obtained at 14.0°C when weight equalled that found for the 29.0°C flies. At 24.0° and 14.0°C this M dc is somewhat

lower than that observed at 29.0°C; at 19.0°C, somewhat higher. In no case is there a wide departure from the 29.0°C value. It cannot be told at present whether the irregularities in the values are of some significance or whether they represent chance deviations from a straight line whose slope may be small and positive, small and negative, or of zero value. Yet it is quite apparent that in *D*, under these experimental conditions the effect of temperature upon the M dc would either be small or non-existent if the flies raised at the different temperatures all had the same average weight.

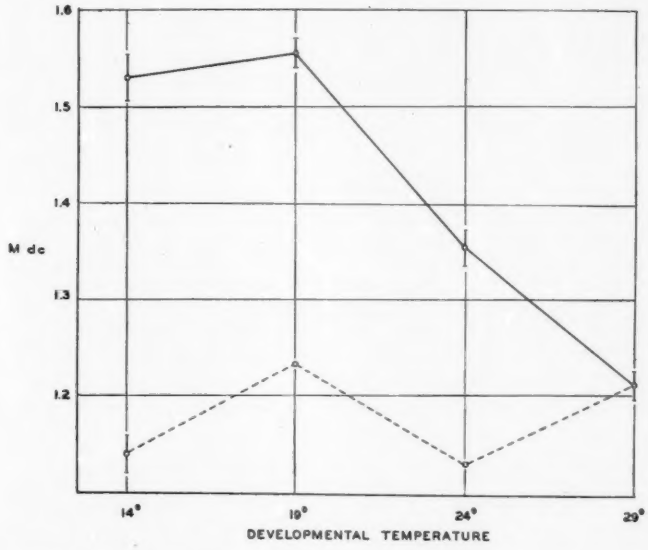

FIGURE 7.—The effect of temperature upon the M dc of *D* flies. The upper, solid-line curve shows the effect of temperature upon the M dc when body size changes at the various temperatures are uncontrolled. The lower, broken line shows the calculated effect of temperature if size were the same at all temperatures. Details in text.

The effect of temperature during the pupal period

PLUNKETT reported that the temperature-effective period in *D* extends over the major portion of larval and pupal life. It was found that when larval life was spent at 24.5°C and pupal at 17.0°C, the M p dc was the same as when larval life passed at 17.0°C and pupal at 24.5°C, that is, "the effect of a higher temperature is the same whether applied to the larval period alone or the pupal period alone" (p. 206). Later work (ROBERTSON 1936, NEEL 1940) has shown that at 24.5°C morphological differentiation

of the bristles in wild-type flies begins early in the second day of pupal development. In view of the difficulty of reconciling this finding with that of PLUNKETT concerning the duration of the temperature-effective period, a repetition of the experiment was undertaken.

Larvae were raised at 19.0° and 29.0°C. When puparium formation began, puparia were collected every four hours. Those formed at 19.0°C were divided into two lots, one of which continued to develop at 19.0°C, and another which completed development at 29.0°C. Similarly, one half of the puparia formed at 29.0°C completed development at this temperature, while the others were placed in the 19.0°C incubator. The results of this

TABLE 6

The effect of temperature during larval and pupal life upon the mean number of dorsocentral bristles per side per fly, in Dichaete males.

LARVAL STAGE AT:	PUPAL STAGE AT:	NUMBER HALF THORACES	M dc $\pm\sigma_m$
19.0°	19.0°	310	1.197±0.019
19.0°	29.0°	206	1.247±0.026
29.0°	19.0°	218	1.000±0.027
29.0°	29.0°	176	0.994±0.030

treatment are shown in table 6. No effect of temperature during the pupal period is evident. This lends further confirmation to the idea of temperature working largely through its effect on size or a factor common to size and M dc. No reason for the discrepancy between these and PLUNKETT's results can be advanced. (The M dc values given here are considerably lower than those given in table 4 for *D* flies raised under optimum food conditions. This is due to the higher larval densities per unit food in these experiments).

DISCUSSION

Differences in the expression of some characteristic from one temperature to another are frequently treated as due to a direct effect of temperature upon some processes resulting in this characteristic, as these are contrasted with other processes affecting the rest of the imago. A temperature increase is thought of as differentially accelerating some one or few of the many organismic reactions taking place during ontogeny. But temperature, whatever else its effects may be, is a size-altering agent. This fact has frequently been overlooked or disregarded in studies of temperature effects, in spite of a large literature on heterogonic growth. It would therefore seem to be a matter of primary importance, wherever a "temperature effect" is involved, to evaluate in so far as possible the relation of size

changes to this effect. In this paper an attempt has been made to do this for two mutants under certain conditions. The Dichaete data, although presenting certain irregularities commented on above (p. 243), indicate a negligible effect of temperature on mutant expression when there is no size change. In the polychaetoid case, analysis shows that only a minor portion of the temperature effect is due to a direct and differential action of temperature upon some bristle-forming processes, and that the role of this direct effect, relative to the total M dc change, is greatest at the low temperatures.

How general is the situation described for these mutants? In a survey of the Drosophila literature, a number of cases were found where the correlations of both size and temperature with the expression of a given character are known. These cases are summarized in table 7. While the list makes no pretense to completeness, it is probably representative. Some of the characters treated there are wild-type and some mutant. The correlation of decreased size with character expression is not always clearly stated as such in the literature. Thus, it is not uncommon to find references to a "starvation effect" or a "crowding effect" (where excess food is not supplied) in character expression. Obviously in these cases decreased size is involved.

In most of the eleven cases listed in the table we find that the characters studied are oppositely correlated with increased size and with increased temperature (attended as the latter is by a size decrease). The two clear exceptions to the general rule are infra-bar and number of teeth in the wild-type sex-comb.

Is this general agreement in the literature a mere coincidence, or is there a causal relation, namely, is the effect of temperature in part at least through the medium of body size or some factors common to size and the characteristic? Certainly an answer to this question cannot be given at present. For none of the cases listed can conclusions be drawn until it is known what effect temperature has when body size is held constant at the various temperatures, and further, what role increase in length of larval life plays. It is to be expected that the part played in the temperature effect by size factors will be found to vary from mutant to mutant, just as it seems to differ for the *pyd* and *D* cases reported here.

A large amount of work has been done towards determining, for various mutants, the time during development at which temperature exerts its effect upon mutant expression. To cite one example, CHILD (1935b) has reported that in the case of the scute-1 mutant, over a temperature range from 18° to 30° "the temperature-effective period in any one fly lies entirely between the time when 89.3 percent and the time when 96.8 percent

TABLE 7

A comparison of the correlation between character expression and a) increased temperature and b) increased body size. The symbol (+) in column 2 indicates that the expression of the normal or mutant character is better (more pronounced) at a high temperature, while a (−) indicates a poorer manifestation at the high temperature. The symbol (+) in column 4 indicates that the wild-type or mutant character is better expressed at large sizes, while (−) indicates poorer manifestation with increasing size.

CHARACTER	HOW CORRELATED WITH INCREASED TEMPERATURE	INVESTIGATOR	HOW CORRELATED WITH INCREASED SIZE	INVESTIGATOR
wild-type wing length	−	ALPATOV and PEARL (1929), ALPATOV (1930), EIGENBRODT (1930), HERSH and WARD (1932), IMAI (1933), STANLEY (1935)	+	ALPATOV (1930), GAUSE (1931)
fore-femur length	−	ALPATOV and PEARL (1929), IMAI (1932), NEEL (unpub.)	+	NEEL (this paper)
number of teeth in wild-type sex-comb	+	COMBS (1937)	+	CASTLE, CARPENTER, CLARK, MAST, and BARROWS (1906)
Bar series of eye shape alleles				
a. Bar and double-bar	+	SEYSTER (1919), KRAFKA (1920), ZELENY (1923), A. H. HERSH (1924), E. C. DRIVER (1926, 1931)	±	MARGOLIS (1935), BODENSTEIN (1939)
b. infra-bar and double infra-bar	−	LUCE (1926), O. W. DRIVER (1931)	−	LUCE (1931)
vestigial wings	−	ROBERTS (1918), HARNLEY (1930), STANLEY (1931), LI and TSUI (1936), RIEDEL (1937)	+	HARNLEY (1930) CHILD (1939)
scute bristles	some bristles increase in frequency, others decrease	CHILD (1935a)	some bristles increase in frequency, others decrease	CHILD (1936)
the *D. funebris* mutants		NEEL (1937)		NEEL (1937)
ascute	+		−	
evaginated	+		−	
interrupted	+		−	
Missing	+		−	

of the egg-larval development has been completed" (p. 154). Other cases where temperature is reported to have an effect only during a brief portion of the egg-larval-pupal life are numerous.

Available evidence indicates that the effects of temperature on size are distributed over a considerable portion of larval life (IMAI 1937). How is this evidence to be reconciled with the usual relative briefness of the temperature-effective period, if a size factor generally plays a role in temperature effects? As a possible answer to this question, capable of experimental tests, it is suggested that both gross size and the expression of a given character may depend upon some such factor as metabolic level. But whereas size is influenced by the metabolic level of the entire larval period, character expression is primarily dependent upon metabolic level at the time of active differentiation of the character. This time may be only a minor portion of the larval period, and would correspond to the temperature-effective period. Changes in the metabolic level at this time would have a much more profound effect upon character expression than on size.

The present work has shown that under a given set of experimental conditions elimination of size changes is attended by a reduction in temperature effects on bristle numbers. It remains to be determined whether this correlation ever breaks down, and if so, to what extent. From the reasoning of the above paragraph it appears possible that there exist periods in development where a decrease in nutritional level would have an effect upon the expression of a given character out of proportion to the effects on gross size.

At the end of the embryonic determination period of any part, the "size-effective" period for that part must come to an end. Accordingly, the exploration of the role of a general size factor in character expression should involve a measurement of larval or pupal size during and at the end of the embryonic determination of the character. This would not only involve working with larvae, but would necessitate an exact knowledge of the determination period. The assumption is therefore implicit in all this work that adult size is proportional to larval size at the time of determination.

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SUMMARY

1. At a constant temperature the expression of the polychaetoid mutant

of *Drosophila melanogaster* is correlated with fly size. This is shown in three ways:

a. When groups of male flies are raised at varying nutritional levels, a double log plot of the mean number of dorsocentral bristles present per side per fly (M dc) against average body weight yields a straight line.

b. In individual flies, the correlation between femur length and the total number of dorso-centrals present on both sides of the fly or the total length of all dorsocentrals present is high.

c. In individual flies, the correlation between weight and total number of dorsocentrals is significant.

2. Increase in length of larval life is not correlated with the M dc of polychaetoid flies.

3. A decrease from 29.0° to 14.0°C in the temperature at which development proceeds results in an increase in the M dc, from 3.058 ± 0.022 to 4.533 ± 0.033. But at the same time there is an increase in mean weight, from 0.784 ± 0.003 to 1.061 ± 0.006 mg.

4. Equations for the regression of log M dc on log mean body weight at the various experimental temperatures employed are established. From these it is shown that under the present experimental conditions the greatest part of the total M dc change observed over the entire temperature range is correlated with the effect of temperature upon imaginal size.

5. In groups of Dichaete males of different average sizes raised at the same constant temperature, the relation between M dc and log average body weight appears to be linear.

6. No evidence is found for a correlation between length of larval life and the M dc of Dichaete flies.

7. When temperature is decreased from 29.0° to 14.0°C, the M dc increases from 1.213 ± 0.015 to 1.530 ± 0.025. However, over the same temperature range average body weight increases from 0.725 to 0.914 mg.

8. It is shown that if the size of Dichaete flies did not change from one temperature to another, there would be no or only a very slight change in the M dc.

9. The application of these findings in the interpretation of other data concerning the effect of temperature upon character expression is discussed.

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